

FORM PTO-1390 (Modified) (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <b>201859US0PCT</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>09/720828</b>
INTERNATIONAL APPLICATION NO. <b>PCT/IB99/01424</b>	INTERNATIONAL FILING DATE <b>16 July 1999</b>	PRIORITY DATE CLAIMED <b>16 July 1998</b>		
TITLE OF INVENTION <b>PEPTIDES OF IL-2 AND DERIVATIVES THEREOF AND THEIR USE AS THERAPEUTIC AGENTS</b>				
APPLICANT(S) FOR DO/EO/US <b>Jacques THEZE, et al.</b>				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
<ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li><input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li><input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li><input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li><input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> <li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li><input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input type="checkbox"/> have been transmitted by the International Bureau.</li> <li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li><input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li><input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li><input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li><input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>				
Items 13 to 20 below concern document(s) or information included:				
<ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li><input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input type="checkbox"/> Certificate of Mailing by Express Mail</li> <li><input checked="" type="checkbox"/> Other items or information:</li> </ol>				
<b>Request for Consideration of Documents Cited in International Search Report</b> <b>Notice of Priority</b>				

U.S. APPLICATION NO. (IF KNOWN) SEE 37 CFR <b>09/720828</b>	INTERNATIONAL APPLICATION NO. <b>PCT/IB99/01424</b>	ATTORNEY'S DOCKET NUMBER <b>201859US0PCT</b>		
21. The following fees are submitted:		<b>CALCULATIONS PTO USE ONLY</b>		
<b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :</b>				
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1,000.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00				
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>		<b>\$860.00</b>		
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).		<input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 <b>\$130.00</b>		
<b>CLAIMS</b>		<b>NUMBER FILED</b>	<b>NUMBER EXTRA</b>	<b>RATE</b>
Total claims	25	- 20 =	5	x \$18.00 <b>\$90.00</b>
Independent claims	4	- 3 =	1	x \$80.00 <b>\$80.00</b>
Multiple Dependent Claims (check if applicable).		<input type="checkbox"/> <b>\$0.00</b>		
<b>TOTAL OF ABOVE CALCULATIONS</b>		<b>= \$1,160.00</b>		
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).		<input type="checkbox"/> <b>\$0.00</b>		
<b>SUBTOTAL</b>		<b>= \$1,160.00</b>		
Processing fee of <b>\$130.00</b> for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).		<input type="checkbox"/> 20 <input type="checkbox"/> 30	+	<b>\$0.00</b>
<b>TOTAL NATIONAL FEE</b>		<b>= \$1,160.00</b>		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).		<input type="checkbox"/> <b>\$0.00</b>		
<b>TOTAL FEES ENCLOSED</b>		<b>= \$1,160.00</b>		
		<b>Amount to be:</b> <b>refunded</b> \$ <b>charged</b> \$ 		
<input checked="" type="checkbox"/> A check in the amount of <b>\$1,160.00</b> to cover the above fees is enclosed.  <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.  <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. <b>15-0030</b> A duplicate copy of this sheet is enclosed.				
<b>NOTE:</b> Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.				
<b>SEND ALL CORRESPONDENCE TO:</b>  <b>22850</b> <b>Surinder Sachar</b> <b>Registration No. 34,423</b>				
 <b>SIGNATURE</b> <b>Norman F. Oblon</b> <b>NAME</b> <b>24,618</b> <b>REGISTRATION NUMBER</b> <b>Jan 16 2001</b> <b>DATE</b>				

201859US-0-PCT

#5  
Rec'd PCT/PTO 05 JUN 2001

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :  
JACQUES THEZE ET AL : ATTN: APPLICATION DIVISION  
SERIAL NO: 09/720,828 :  
FILED: JANUARY 16, 2001 :  
FOR: PEPTIDES OF IL-2 AND :  
DERIVATIVES THEREOF AND :  
THEIR USE AS THERAPEUTIC :  
AGENTS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Responsive to the Office Communication dated May 5, 2001, Applicants submit herewith a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Page 35, after the last line, beginning on a new page, please replace the original Sequence Listing with the substitute Sequence Listing attached herewith.

REMARKS

Claims 1-25 are active in the present application. Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants further submit that an executed Declaration properly identifying the application was filed on March 1, 2001. Accordingly, Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.



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**Marked-Up Copy**  
Serial No: 09/720,828  
Amendment Filed on:  
June 5, 2001

IN THE SPECIFICATION

Page 35, after the last line, beginning on a new page, please replace the original Sequence Listing with the substitute Sequence Listing attached herewith.

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SEQUENCE LISTING

<110> THEZE, JACQUES

ECKENBERG, RALPH

MOREAU, JEAN-LOUIS

GOLDBERG, MICHEL

ROSE, THIERRY

ALZARI, PEDRO

MAZIE, JEAN-CLAUDE

<120> NEW PEPTIDES OF IL-2 AND DERIVATIVES THEREOF AND THEIR USE AS THERAPEUTIC AGENTS

<130> 201859US0PCT

<140> 09/720,828

<141> 2001-01-16

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<151> 1999-07-16

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20 25 30

093200828-036103

PEPTIDES OF IL-2 AND DERIVATIVES THEREOF AND THEIR USE AS THERAPEUTIC AGENTS

The present invention relates to new peptides of IL-2, and derivatives thereof and their use as therapeutic agents.

5 Interleukin-2 (IL-2) is the main growth factor of T lymphocytes (THÈZE et al. 1996, *Immunol. Today* 17:481-486). By regulating T helper lymphocyte activity IL-2 increases the humoral and cellular immune responses. By stimulating cytotoxic CD8 T cells and NK cells this cytokine participates to the defense mechanisms against tumors and viral infections. IL-2 is used in therapy against metastatic melanoma  
10 and renal adenocarcinoma. IL-2 is used in clinical trials in many forms of cancer (LOTZE and ROSENBERG 1988, Interleukin 2 as a Pharmacologic Reagent. in *Interleukin 2*, K.A. Smith, Academic Press: p. 237-89). It is also used in HIV infected patients and leads to a significant increase in CD4 counts (KOVACS et al. 1996, *New Engl. J. of Medicine* 335:1350-6).

15 Human IL-2 is a protein of 133 amino acids (aa) composed of four  $\alpha$  helices connected by loops of various length; its tridimensional structure has been established. IL-2R is composed of three chains  $\alpha$ ,  $\beta$  and  $\gamma$ . IL-2R $\alpha$  controls the affinity of the receptor. IL-2R $\beta$  and IL-2R $\gamma$  are responsible for IL-2 signal transduction. The different molecular areas of IL-2 interacting with the three chains of the IL-2 R have been  
20 defined. More specifically it has been determined that  $\alpha$  helix A as well as the NH<sub>2</sub> terminal area of IL-2 (residues 1 to 30) control the interactions IL-2/IL-2R $\beta$  (ECKENBERG et al. 1997, *Cytokine* 9:488-98) : IL-2R $\beta$  chain is the most important in IL-2 signaling (THÈZE et al. 1990).

25 The effects of human interleukin-2 (IL-2) on its target cells are mediated through specific cell surface receptors (IL-2R) (TANIGUCHI et al. (1983) Nature 302:305-310; ROBB et al. (1984) Proc. Natl. Acad. Sci. USA 81:6486-6490; SMITH KA. 1988a. Interleukin-2; SMITH KA (1988b) Science 240:1169-1176). IL-2R comprises at least three subunits encoded by different genes (MINAMI et al. (1993) Annu. Rev. Immunol. 11:245-267; TANIGUCHI et al. (1993) Cell 73:5-8). The first  
30 component to be identified, IL-2R $\alpha$ , is a 55 kDa protein that binds IL-2 with a Kd of  $\approx$  10 nM (UCHIYAMA et al. (1981) J. Immunol. 126:1293-1297; LEONARD et al. (1984) Nature 311:626-631). The role of IL-2R $\alpha$  (KUMAR et al. (1987) J. Immunol. 139:3680-3684) and the influence of IL-2 on IL-2R $\alpha$  gene expression have been studied (BISMUTH et al. (1985) Eur. J. Immunol. 15:723-727; FROUSSARD et al. (1991) Mol.  
35 Immunol. 28:87-93). The second IL-2R component, IL-2R $\beta$  is a 75 kDa protein with a large intracytoplasmic domain (286 aa) (TESHIGAWARA et al. (1987) J. Exp. Med.

165:223-238; HATAKEYAMA et al. (1989) Science 244:551-556; TSUDO et al. (1989) Proc. Natl. Acad. Sci. USA 86:1982-1986). The last component to be identified, IL-2R $\gamma$ , is a 64 kDa protein (TAKESHITA et al. (1992) Science 257:379-382; ISHII et al. (1994) Int. Immunol. 6:1273-1277). IL-2R $\beta$  and IL-2R $\gamma$  belong to the hematopoietin receptor family whereas IL-2R $\alpha$  belongs to another family of molecules (THÈZE J (1994) Eur. Cytokine Netw. 5:353-368). In the mouse system all three chains are required to form a functional receptor (MOREAU et al. (1995a) J. Immunol. 155:3401-3408; CHASTAGNER et al. (1996) Eur. J. Immunol. 26:201-206). In the human system two receptors are functional. When associated, human IL-2R $\beta$  plus IL-2R $\gamma$  form an intermediate affinity receptor with a Kd of  $\sim$ 1 nM, whereas expression of all three chains leads to the formation of a high affinity IL-2R (Kd  $\sim$ 10 pM).

The structure of IL-2 (MACKAY D (1992) Science 257:410-413) is composed of a compact core bundle of four antiparallel  $\alpha$  helices connected by three loops (Figure 1). Some of the interactions between IL-2 and IL-2R $\alpha$  (SAUV et al. (1991) Proc. Natl. Acad. Sci. USA 88:4636-4640; WANG et al. (1995) Eur. J. Immunol. 25:1212-1216) and IL-2R $\gamma$  subunits (Voss et al. (1993) Proc. Natl. Acad. Sci. USA 90:2428-2432; Buchli et al. (1993) Arch. Biochm. Biophys. 307:411-415) have been elucidated, but less is known concerning IL-2/IL-2R $\beta$  interaction, despite the fact that IL-2R $\beta$  chain plays a very critical role in signal transduction (TANIGUCHI T (1995) Science 268:251-255).

It has been shown that one substitution Asp20 by Lys (mutant D20K) prevents binding to IL-2R $\beta$  (COLLINS et al. (1988) Proc. Natl. Acad. Sci. USA 85:7709-7713). In a recent report the role of the sequence (Leu17, Leu18, Leu19, Asp20, Leu21) from IL-2  $\alpha$  helix A, in IL-2/IL-2R $\beta$  interactions was analyzed by cassette mutagenesis (BERNDT et al. (1994) Biochemistry 33:6571-6577). However the data were difficult to interpret since most of the proteins produced have multiple mutations inside and outside of the sequence of interest. Only one analog with a single mutation was studied (L21V). More surprisingly it was reported in this study that deletion of the segment spanning residues 17-31 (Del1) gives a protein with full agonist activity.

IL-2 peptides and derivatives were described in *Cytokine* (1997) 7:488-498, but were not tested in an *in vitro* system for biochemical activity such as cytokine activity, and in particular for IL-2-like activity.

In view of the aforementioned deficiencies attendant with the prior art analysis of IL-2 agonists and antagonists, as well as with methods of modulating IL-2 activity therewith, it is clear that there exists a need in the art for the same.

Accordingly, one object of this invention is to provide compositions having an IL-2-like activity and methods for their use as therapeutic agents. The applications of such recombinant, synthetic or hybrid peptides are thus one object of the invention. These compositions are defined as having the following characteristics: a) containing one or more peptides at least five amino acids in length; and b) inhibiting or mimicing the binding of helix A of interleukin-2 (IL-2) to a subunit of an IL-2 receptor (IL-2R).

Another object of the invention is the use of a purified peptide having the following characteristics: a) the peptide is at least five amino acids in length; b) the peptide binds to a subunit of an IL-2 receptor (IL-2R); and c) the peptide induces phosphorylation of the subunit of the IL-2R.

A further object of the invention concerns the preparation of the antibodies which recognize the peptides of the invention, and the therapeutic use of these antibodies.

A further object of the invention is the use of DNA sequences encoding the peptides of the invention and their derivatives. Such DNA fragments are useful for gene therapy among other applications. The use of a DNA of the following sequence  
ATG GCT CCG ACG AGC AGC TCC ACC AAG AAA ACC CAG CTC CAG CTC GAA  
CAC CTG CTG CTG GAC CTG CAG ATG ATC CTG AAC GGT ATC AAC AAC  
(SEQ ID NO.: 1) or said SEQ ID NO.: 1 without the first codon ATG (SEQ ID NO.: 3) is one particular object of the invention.

Yet another object of the invention is to provide a method for detecting the activity of an IL-2-like peptide, wherein the IL-2 activity is measured by the binding of the IL-2R to the peptide having the IL-2 agonist or antagonist activity. A still further object of the invention is the use of compounds which inhibit the activity of an IL-2R by contacting the IL-2R with an amount of the selected antagonist peptide sufficient to inhibit binding of IL-2 to the IL-2R under conditions that allow binding of the peptide to the IL-2R to occur.

Another object of the invention is to provide a method for the selection of antibodies specific for the purified peptide with IL-2-like activity as defined herein. These monoclonal or polyclonal antibodies can inhibit binding of IL-2 to the IL-2R under conditions that allow binding of the peptide to the IL-2R to occur. The therapeutic use of these antibodies is also a part of the present invention. In particular, these antibodies specific for the purified peptides are useful for treating or preventing undesirable immune reactions such as graft rejection or autoimmune disorders, for example, rheumatoid arthritis.

A still further object of the invention is to provide a method for inducing in a patient the biological effects of IL-2 by administering to the patient an amount of the agonist peptide of the invention sufficient to induce those biological effects, or by administering a combination of various cytokines and purified peptide. By 5 various cytokines is meant for example IL-4, IL-9, IL-15 or IL-2.

Another object of the invention relates to the nucleic acid sequences corresponding to the amino acid sequence of the purified peptide and its derivatives according to the invention. A preferred embodiment is the nucleotide sequence encoding the purified peptide IP130 having the following sequence (SEQ ID NO.: 2):

10 Met Ala Pro Thr Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu  
His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn  
or a sequence which does not comprise the first Met (SEQ ID NO.: 4).

15 This sequence or a sequence derived therefrom can be inserted in an appropriate vector capable of expressing the product *in vivo*, in a bacterium or in a eukaryotic cell, particularly in yeast or a mammalian cell. These constructs are useful for gene therapy among other uses.

20 With the foregoing and other objects, advantages and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the preferred embodiments of the invention and to the appended claims.

25 A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Figure 1 shows a schematic representation of the human IL-2 structure. The protein contains 133 amino-acid residues (molecular weight: 15-18 kDa, depending on the degree of glycosylation). Four  $\alpha$ -helices, denoted A (residue positions 6-29), B-B' (positions 53-72), C (positions 81-97), and D (positions 113-133) surround a central hydrophobic core. Residues Leu17 and Asp20 (see text) occur in the N-terminal helix A. The structure of the loop between  $\alpha$  helix C and  $\alpha$  helix D was undetermined. Atomic coordinates were obtained from the Brookhaven Protein Data Bank (BERNSTEIN et al. (1977) J. Mol. Biol. 112:535-542) entry link, deposited by D.B. MCKAY (MACKAY D (1992) Science 257:410-413). The figure was drawn with the program Molscript (KRAULIS (1991) J. Appl. Crystallogr. 24:946-950).

35 Figure 2 shows the binding and binding inhibition of mAb H2-8. Binding experiments: plates were coated with IL-2, peptide 1-22 or peptide 1-30. Control

is represented by non coated plates. Binding of mAb was revealed with alkaline phosphatase goat anti-mouse polyvalent Ig conjugate. Inhibition experiments: concentrations of mAbs H2-8 or 19B11 giving  $\frac{1}{2}$  maximal binding on IL-2 coated plates were used. These dilutions were mixed for 1 hr at 37°C with the indicated concentration of inhibitors before addition to wells coated by IL-2.

Figure 3 shows the binding of mAb H2-8 on peptide 1-30. Plates were coated with mAb H2-8 and were incubated with peptide 1-30 as described.

Figure 4 shows the biological effects of mAb H2-8 on IL-2 Pro<sup>125</sup>. Different concentrations of IL-2 Pro<sup>125</sup> were tested on the proliferation of TS1 $\beta$  cells (IL-2R $\alpha^-$ , human IL-2R $\beta^+$ , mouse IL-2R $\gamma^+$ ). The proliferation was measured by [<sup>3</sup>H]TdR incorporation as indicated.

Various concentrations (indicated in parenthesis -  $\mu$ g/ml) of mAb H2-8 were tested. As control the absence of effects of mAb H2-8 on IL-2 dependent proliferation of TS1 $\beta$  cells was verified.

Figure 5 shows the model of IL-2/IL-2R interactions. Figure 8(A) Position of residues Leu17 and Asp20 in the IL-2 structure with respect to helices A, C and D, in a view perpendicular to the axis of helix A. Figure 8(B) Position of residue Leu17 with respect to helices A and B-B'. The side chain of Leu17 is located in a leucine-rich hydrophobic core of the molecule. The charged side chain of Asp20 is partly exposed to solvent. The two orientations of the molecule shown here are roughly perpendicular to that shown in Figure 1.

Figure 8(C) The model of the IL-2/IL-2R complex (BAMBOROUGH et al. (1994) Structure 2:839-851) is based on the structure of the human growth hormone and its receptor (DE VOS et al. (1992) Science 255:306-312). For clarity, only  $\alpha$ -helices A and D of IL-2 and the  $\beta$  and  $\gamma$  chains of the receptor are shown. The IL-2 positions studied, Leu17, Asp20 and residues Arg15 and Tyr134 of IL-2R $\beta$  are labelled. Positions Cys125 and Ser127 are also shown. Secondary structural elements as defined by the program DSSP (KABSCH et al. (1983) Biopolymers 22:2577-2637). Atomic coordinates of the complex were obtained from the Brookhaven Protein Data Bank, entry code film.

Figure 6 is a schematic representation of IL-2 /IL-2R interactions and the IP130 sequence (SEQ ID NO.: 4). IL-2 receptor is composed of three subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ). (See Imm. Today, 1996).

Figure 7 demonstrates that IP130 induces proliferation and acts in synergy with IL-2. Proliferative activity was tested on TS1 $\beta$ 2 (grown in IL-2). Background of  $1.4 \times 10^3$  cpm was subtracted. Synergy with IL-2 is also observed. TS1 $\beta$

target cells are derived from TS1 murine cells (which only express murine IL-2R $\gamma$ ), after transfection with the human IL-2R $\beta$  gene.

Figure 8 shows that the proliferation induced by IP130 is not due to synergy with residual growth factor coming from the culture medium. TS1 $\beta$  cells, used in this study, are cultured in IL-4. TS1 $\beta$  proliferates with IP130 and this proliferation is not inhibited by 11B11 mAB, which neutralizes proliferation induced by IL-4. Figure 8A shows IP130 proliferative activity. Figure 8B shows IL-4 proliferative activity. Figure 8C shows IP130 + mAB 11B11 (anti-IL-4). Figure 8D shows IL-4 + mAB 11B11 ( $\square$ ) and IL-4 + mAB 145 (control mAB) ( $\blacktriangledown$ ). Proliferative activity was tested on TS1 $\beta$ 4 (grown in IL-4).

Figure 9 demonstrates that human IL-2R $\beta$  is essential for the proliferation induced by IP130. Figure 9A shows IP130 activity on TS1 cells transfected with human IL-2R $\beta$ . Figure 9B shows the effect of anti-human IL-2R $\beta$  neutralizing antibody (A41) on IP130 activity. TS1 cells only proliferate after transfection by the human IL-2R $\beta$  gene. As for IL-2, murine IL-2R $\beta$  chain does not allow proliferation in the presence of IP130. TS1 $\beta$  proliferation induced by IP130 is specifically neutralized by the mAB A41 (anti human IL-2R $\beta$ ).

Figure 10 is a summary of IP130 (SEQ ID NO.: 4) and of derivative molecule's structure-function studies. A family of peptides was studied for helicity, oligomerization and biologic activity (see also general presentation of the data).

Figure 11 is a model of IP130/IL-2R $\beta$  interactions. IP130 is tetrameric and IL-2R $\beta$  forms a dimer in solution. Proposed from the results obtained with the three dimensional structure of IL-2 and growth hormone/receptor complex.

Figure 12 shows the pattern of phosphorylation induced by IP130 and involvement of SHC. IP130 induces SHC protein phosphorylation. The two bands corresponding to SHC isoforms are phosphorylated after a ten minute stimulation by IP130. Kinetics of Shc phosphorylation is shown on the left. A Western blot of Shc protein is shown on the right.

Figure 13 is an electrophoretic mobility shift assay which shows that IP130 does not induce STAT activity. STAT activation is analyzed in KIT225 cells nuclear extracts after stimulation by IL-2, IP130 or IL-2 + IP130. Only cells stimulated by IL-2 or IL-2 + IP130 show a STAT activation.  $\beta$ -casein probes were used in the study. The same results were obtained with two other probes (GAS and GIRE).

Figure 14 depicts a model of signal transduction and IP130. IL-2 uses three main pathways: 1°/JAK/STAT depending on IL-2R $\beta$  $\gamma$  complex; 2°/SHC/MAPK

initiated on the phosphorylated IL-2 R $\beta$  chain and 3°/PI3K. In accordance with the (IP130)<sub>4</sub>/(IL-2R $\beta$ )<sub>2</sub> model (Figure 14), IP130 does not induce the JAK/STAT pathway but induces the SHC/MAPK pathway. (JAK: janus activated kinase; PI3K: phosphatidyl inositol, 3-phosphate kinase; STAT: signal transducers and activators of transcription; MAPKK: mitogen activated protein kinase; MAPK: mitogen activated protein kinase; ↗: induction of transcription).

Figure 15 shows the cell cycle entry (S+G2/M) of IP130 stimulated NK cells. PBMC are stimulated by IL-2, IP130 or IL-2 + IP130. Non-specific responses (J+1 in medium) are subtracted. NK cells entry into S+G2/M phases are measured by propidium iodide and analyzed with the ModFit 2.0 software (Becton Dickinson).

Figure 16 shows that IP130 stimulates LAK activity. In this experiment, the kinetics of LAK activity stimulation has been studied. Histograms show the results with an effector/target ratio of ten.  $\Delta\%$  lysis = lysis induced by IL-2 and/or IP130 - spontaneous lysis.

The present invention relates to the use of IL-2 peptides derived from interleukin-2 (IL-2), for their therapeutic use in mammals, and particularly in humans. The peptides are selected from fragments of IL-2 and derivatives of IL-2. The derivatives are defined as containing an amino acid sequence capable of binding to the IL-2R $\beta$  chain under the conditions described herein, or capable of binding to the monoclonal antibodies produced by H2-8 hybridoma. The invention also relates to antibodies directed against the peptides according to the invention which likewise mimic and/or modulate IL-2 activity. The diagnostic and therapeutic approaches involve the use of the purified peptides and the antibodies for detecting and/or modulating IL-2 binding to IL-2R *in vitro* and *in vivo*. In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., SAMBROOK et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994)]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

The terms "IL-2 peptide," "IL-2 agonist/antagonist," "IP130/IP130 derivatives," and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application. These terms refer to proteinaceous material including single or multiple proteins or recombinant product or peptides obtained by chemical synthesis, and extend to those proteins having the amino acid sequence data described herein and presented in Figure 10 (SEQ ID NO.: 3). The profile of biological activities set forth hereinafter is one of the aspects of the present application.

Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms "IL-2 peptide," "IL-2 agonist/antagonist" and "IP130/IP130 derivatives or purified peptide(s)" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property or immunoglobulin-binding is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues are shown in the following Table of Correspondence:

25

TABLE OF CORRESPONDENCE

SYMBOL	AMINO ACID		
	1-Letter	3-Letter	
	Y	Tyr	tyrosine
	G	Gly	glycine
30	F	Phe	phenylalanine
	M	Met	methionine
	A	Ala	alanine
	S	Ser	serine
	I	Ile	isoleucine
35	L	Leu	leucine
	T	Thr	threonine

V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
5	Q	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
10	N	asparagine
C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide," is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA 5 may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing 10 the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) 15 of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., 20 MANIATIS et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

It should be appreciated that also within the scope of the present invention are the biological uses of the DNA sequences encoding IL-2 peptides having the same amino acid sequence as IP130 (SEQ ID NO.: 2 or SEQ ID NO. :4), but which 25 are degenerate to the DNA encoding SEQ ID NO.: 2 or SEQ ID NO.: 4. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

Phenylalanine (Phe or F)	UUU or UUC
Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
Isoleucine (Ile or I)	AUU or AUC or AUA
Methionine (Met or M)	AUG
Valine (Val or V)	GUU or GUC or GUA or GUG
Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
Proline (Pro or P)	CCU or CCC or CCA or CCG
Threonine (Thr or T)	ACU or ACC or ACA or ACG

	Alanine (Ala or A)	GCU or GCG or GCA or GCG
	Tyrosine (Tyr or Y)	UAU or UAC
	Histidine (His or H)	CAU or CAC
	Glutamine (Gln or Q)	CAA or CAG
5	Asparagine (Asn or N)	AAU or AAC
	Lysine (Lys or K)	AAA or AAG
	Aspartic Acid (Asp or D)	GAU or GAC
	Glutamic Acid (Glu or E)	GAA or GAG
	Cysteine (Cys or C)	UGC or UGC
10	Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
	Glycine (Gly or G)	GGU or GGC or GGA or GGG
	Tryptophan (Trp or W)	UGG
	Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences. The corresponding codons for DNA have a T substituted for U.

Modifications of the peptides can be made in the DNA encoding SEQ ID NO.: 2 or SEQ ID NO.: 4 such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

The following is one example of various groupings of amino acids:

Amino acids with nonpolar R groups

- Alanine
- Valine
- 35 Leucine
- Isoleucine

Proline  
Phenylalanine  
Tryptophan  
Methionine

5    Amino acids with uncharged polar R groups

Glycine  
Serine  
Threonine  
Cysteine

10   Tyrosine  
Asparagine  
Glutamine

Amino acids with charged polar R groups (negatively charged at Ph 6.0)

Aspartic acid  
15   Glutamic acid

Basic amino acids (positively charged at pH 6.0)

Lysine  
Arginine  
Histidine (at pH 6.0)

20   Another grouping may be those amino acids with phenyl groups:  
Phenylalanine  
Tryptophan  
Tyrosine

Another grouping may be according to molecular weight (i.e., size of R groups):

25	Glycine	75
	Alanine	89
	Serine	105
	Proline	115
	Valine	117
30	Threonine	119
	Cysteine	121
	Leucine	131
	Isoleucine	131
	Asparagine	132
35	Aspartic acid	133

Glutamine	146
Lysine	146
Glutamic acid	147
Methionine	149
5 Histidine (at pH 6.0)	155
Phenylalanine	165
Arginine	174
Tyrosine	181
Tryptophan	204

10 Particularly preferred conservative substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH<sub>2</sub> can be maintained.

15 Amino acid substitutions may also be introduced in IL-2 or peptides thereof to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because 20 of its particularly planar structure, which induces β-turns in the protein's structure.

The biologically active peptides of the invention preferably encompass a region of the IL-2 sequence which includes amino acids 17-20, although one or more of these amino acid residues may be substituted with another amino acid, or a modified amino acid. The use of the preferred peptide containing at least 5 amino acids in length, 25 more preferably 8-12 amino acids, and most preferably at least 15 amino acids in length is one aspect of the invention, as well as the use of the peptide of the invention based on amino acids 1-30 of IL-2.

30 Biological or physiological activity of IL-2 may be considered to include the stimulation of CD4, CD8 and NK cells, and may include antiviral and antitumor activities. Biological or physiological activity of IP130 and other peptides of the invention may include the foregoing activities of IL-2, as well as induction of SHC phosphorylation and induction of the SHC/MAPK pathway.

35 A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian

genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-  
5 occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in  
10 U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as  
15 used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab,  
20 Fab', F(ab')<sub>2</sub> and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and F(ab')<sub>2</sub> portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from F(ab')<sub>2</sub> portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.  
25

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric)  
35 monoclonal antibody.

Preferred antibodies of the present invention bind to the peptides of the invention, described above. The antibodies which bind the peptides of the invention may be used as diagnostic agents for analyzing IL-2 binding to its receptor, and may also be used as therapeutic agents, to enhance or inhibit the binding of IL-2 to its receptor. In a 5 preferred embodiment, the antibody of the invention inhibits the binding of IL-2 and/or IP130 to the IL-2R.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when 10 administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in a feature of pathology such as for example, elevated blood pressure, 15 fever or white cell count as may attend its presence and activity.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and 20 maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such 25 "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization 30 conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined  $T_m$  with washes of higher stringency, if desired.

In its primary aspect, the present invention concerns the use of IL-2 peptides which modulate IL-2 activity. By "modulate" is meant either agonist or antagonist activity which either increases or suppresses the physiological effects of IL-2, such as the proliferation of cells, as described below.

5 As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes an IL-2 peptide, or a fragment thereof, that possesses a molecular weight preferably of about 2-5 kD and an amino acid sequence set forth in Figure 10 (SEQ ID NO.: 4) or a sequence wherein SEQ ID NO.: 4 is modified by insertion, deletion and/or substitution; preferably  
10 a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the peptide has a nucleotide sequence, is complementary to, or hybridizes under standard hybridization conditions to a DNA sequence encoding SEQ ID NO:2 or SEQ ID NO.: 4.

15 The possibilities both diagnostic and therapeutic that are raised by the existence of the IL-2 peptides, derive from the fact that the peptides appear to participate in direct and causal protein-protein interaction between the IL-2 peptide and the IL-2 receptor, specifically IL-2R $\beta$ , and those factors that thereafter mediate cellular events. In particular the IP130 peptide has been shown to induce phosphorylation of IL-2R $\beta$ , to induce c-myc; and to induce natural killer (NK) cells to enter the cell cycle. As suggested earlier and elaborated further on herein, the present invention contemplates pharmaceutical intervention in the cascade of reactions in which the IL-2R is implicated, to modulate the activity initiated by IL-2 and peptides thereof.

20 Thus, in instances where it is desired to reduce or inhibit the IL-2 induced activity, an appropriate IL-2 peptide inhibitor of IL-2 could be introduced to block the interaction of the IL-2 with the IL-2R. Correspondingly, instances where insufficient IL-2 induced activity is taking place could be remedied by the introduction of additional quantities of the appropriate IL-2 peptide agonist, such as IP130, or its chemical or pharmaceutical cognates, analogs, fragments and the like.

25 As discussed earlier, the IL-2 peptides or their binding partners or other ligands or agents exhibiting either mimicry or antagonism to IL-2 or control over its production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated with undesirable levels of IL-2 for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the IL-2 peptides or their subunits may  
30  
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vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of IL-2 and/or peptides thereof may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring IL-2 receptor activity or the like. For example, IL-2 or peptides thereof may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic or antagonize the activity(ies) of the IL-2 peptides of the invention may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. SCHREIER et al., "Hybridoma Techniques" (1980); HAMMERLING et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); KENNEDY et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against IL-2 peptides can be screened for various properties; i.e., isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that modulate the activity of IL-2 or peptides thereof. Such monoclonals can be readily identified in cellular proliferation assays. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant IL-2 or IL-2 peptides is possible.

Preferably, the anti-IL-2 antibody used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-IL-2 antibody molecules used herein be in the form of Fab, Fab', F(ab')<sub>2</sub> or F(v) portions of whole antibody molecules.

A diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of a labeled IL-2 peptide or an antagonist thereof, such as an anti-IP130 antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-IL-2 antibody molecules used herein be in the form of Fab, Fab',

F(ab')<sub>2</sub> or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefitting from this method include those suffering from cancer, a pre-cancerous lesion, a viral infection or other like pathological derangement. Methods for isolating the IL-2 peptide and inducing anti-IL-2 antibodies and for determining and 5 optimizing the ability of anti-IL-2 antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or F(ab')<sub>2</sub> portions of useful antibody molecules, can be 10 prepared using the hybridoma technology described in *Antibodies - A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference. Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized 15 with an IL-2 peptide or IL-2 R-binding portion thereof.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their 20 ability to immunoreact with the present IL-2 mutant or peptide and their ability to inhibit specified IL-2 activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for 25 the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred 30 mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; DULBECCO et al., *Virol.* 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-IL-2 antibodies are also well-known in the art. See NIMAN et al., *Proc. Natl. Acad. Sci. USA*, 80:4949-4953 (1983). Typically, the present IL-2 peptide or a peptide analog is used either alone or conjugated 35

to an immunogenic carrier, as the immunogen in the before described procedure for producing anti-IL-2 monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the IL-2 mutant or peptide analog.

The present invention further contemplates the use of therapeutic compositions which are useful in practicing the therapeutic methods of this invention. In one embodiment, the therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a IL-2 peptide, a purified peptide or a derivative thereof or a polypeptide analog thereof or fragment thereof, as described herein as an active ingredient. In a preferred embodiment, the therapeutic composition comprises an active compound containing a purified peptide capable of modulating the specific binding of the present IL-2 with the IL-2R.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

The use of the compositions may be by administration in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of IL-2 binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The use of the therapeutic compositions may be by administration in a composition which further includes an effective amount of the IL-2 agonist/antagonist or analog thereof, and one or more of the following active ingredients: an antibiotic, a steroid.

5 As used herein, "pg" means picogram, "ng" means nanogram, "ug" or " $\mu$ g" mean microgram, "mg" means milligram, "ul" or " $\mu$ l" mean microliter, "ml" means milliliter, "l" means liter.

10 Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

15 Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

20 A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col E1, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage  $\lambda$ , e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2 $\mu$  plasmid or derivatives thereof; vectors useful in eukaryotic 25 cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

30 Any of a wide variety of expression control sequences - - sequences that control the expression of a DNA sequence operatively linked to it - - may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage  $\lambda$ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic 35 enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast  $\alpha$ -

mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and 5 prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences 10 and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the 15 host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting an expression control sequence, a variety of factors will 20 normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA 25 sequences to be expressed, and the ease of purification of the expression products.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

30 It is further intended that for the therapeutic use of the peptides according to the invention, the IL-2 peptide analogs may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of IL-2 material. Other analogs, such as muteins, can be produced by standard 35 site-directed mutagenesis of IL-2 coding sequences. Analogs exhibiting "IL-2 activity"

such as small molecules, whether functioning as promoters or inhibitors, may be identified by known *in vivo* and/or *in vitro* assays.

As mentioned above, a DNA sequence encoding IL-2 peptides can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the IL-2 peptide amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., EDGE, *Nature*, 292:756 (1981); NAMBAIR et al., *Science*, 223:1299 (1984); JAY et al., *J. Biol. Chem.*, 259:6311 (1984).

Synthetic DNA sequences allow convenient construction of genes which will express IL-2 peptide analogs or "muteins". Alternatively, DNA encoding muteins can be made by site-directed mutagenesis of native IL-2 genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, *Science*, 244:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

In accordance with the gene therapy applications of the present invention, the preparation of antisense oligonucleotides and ribozymes may be used to modulate the expression of IL-2 at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See WEINTRAUB, 1990; MARCUS-SEKURA, 1988.) In the cell, they hybridize to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into cells. Antisense methods have been used to inhibit the expression of many genes *in vitro* (MARCUS-SEKURA, 1988; HAMBOR et al., 1988).

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for IL-2 or molecules which stimulate or reduce IL-2 secretion and their ligands.

The present invention also relates to a variety of diagnostic applications, including methods for detecting IL-2 presence and activity, by reference to their ability to elicit the activities which are mediated by the present IL-2 peptides. As mentioned earlier, the IL-2 peptide can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence of particular IL-2 and/or IL-2 R activity in suspect target cells.

As described in detail above, antibody(ies) to the IL-2 peptides can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to the IL-2 peptide will be referred to herein as Ab<sub>1</sub>, and antibody(ies) raised in another species as Ab<sub>2</sub>.

The presence of IL-2 and IL-2 peptides in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the IL-2 peptide labeled with a detectable label, antibody Ab<sub>1</sub> labeled with a detectable label, or antibody Ab<sub>2</sub> labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "ILP" stands for the IL-2 peptide:

- A. ILP\* + Ab<sub>1</sub> = ILP\*Ab<sub>1</sub>
- B. ILP + Ab<sub>1</sub>\* = ILPAb<sub>1</sub>\*
- C. ILP + Ab<sub>1</sub> + Ab<sub>2</sub>\* = ILPAb<sub>1</sub>Ab<sub>2</sub>\*

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody," or "DASP" procedure.

In each instance, the ILP forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab<sub>2</sub> is that it will react with Ab<sub>1</sub>. This is because Ab<sub>1</sub> raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab<sub>2</sub>. For example, Ab<sub>2</sub> may be raised in goats using rabbit antibodies as antigens. Ab<sub>2</sub> therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab<sub>1</sub> will be referred

to as a primary or anti-ILP antibody, and Ab<sub>2</sub> will be referred to as a secondary or anti-Ab<sub>1</sub> antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

The IL-2 peptide or its binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, <sup>36</sup>Cl, <sup>51</sup>Cr, <sup>57</sup>Co, <sup>58</sup>Co, <sup>59</sup>Fe, <sup>90</sup>Y, <sup>125</sup>I, <sup>131</sup>I, and <sup>186</sup>Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase,  $\beta$ -glucuronidase,  $\beta$ -D-glucosidase,  $\beta$ -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system which can be utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

A further embodiment of this invention is the diagnostic application of commercial test kits suitable for use by a medical specialist. The kit may be prepared to determine the presence or absence of predetermined IL-2R activity or predetermined IL-2 activity capability in suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled IL-2 peptide or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined IL-2 R activity, comprising:

- 5 (a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the present IL-2 peptide factor or a specific binding partner thereto, to a detectable label;
- (b) other reagents; and
- (c) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

- 10 (a) a known amount of the IL-2 peptide as described above (or a binding partner) generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;
- (b) if necessary, other reagents; and
- (c) directions for use of said test kit.

15 In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

- (a) a labeled component which has been obtained by coupling the IL-2 peptide to a detectable label;
- 20 (b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:
- (i) a ligand capable of binding with the labeled component (a);
- (ii) a ligand capable of binding with a binding partner of the labeled component (a);
- 25 (iii) a ligand capable of binding with at least one of the component(s) to be determined; and
- (iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and
- 30 (c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the IL-2 peptide and a specific binding partner thereto.

35 Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

## EXAMPLES

### Example 1: Characterization of mouse monoclonal antibody H2.8

Female BALB/c mice were repeatedly immunized with 25 to 50 µg of peptide 1-30 per injection. The peptide was coupled to the KLH carrier and injected with 5 Complete Freund's adjuvant (first injection) or incomplete Freund's adjuvant (subsequent injections). The titer of the anti-IL-2 activity was assessed in a group of five animals. Spleen cells from the animal giving the best response were used for fusion with cell line SP2-0. Four hybridomas with specific anti-IL-2 activity were cloned. The mAbs were purified from the corresponding ascitic fluid by ammonium sulfate precipitation. The purity 10 of the reagents (> 80%) was verified by polyacrylamide gels. The properties of the mAbs were characterized. The results are reported only for mAb H2-8. The isotype (IgG1) and the Kd ( $1.4 \times 10^{-9}M$ ) of mAb H2-8 were determined.

Mouse mAbs 19B11 (IgG1) and 2C4 (IgG1) previously characterized (MOREAU et al (1995b) Mol. Immunol. 32:1047-1056; REBOLLO et al (1992) Mol. 15 Immunol. 29:119-130) were used as controls. mAbs 19B11 and 2C4 inhibit the binding of IL-2 to IL-2R $\beta$  and recognize the peptides 1-10 (see below), 1-22 and 1-30. Rat monoclonal 11B11 (IgG, k) specific for murine IL-4 was provided by Dr. W. PAUL (National Institute of Health, Bethesda MD, USA and used as previously described (MOREAU et al. (1995b) Mol. Immunol. 32:1047-1056).

20 The inhibitory effect of purified mAb H2-8 was first assayed on the binding of  $^{125}\text{I}$ -labeled IL-2. Its effects were measured on two transfecants derived from a mouse cell line expressing only mIL-2R $\gamma$ . Transfectant TS1 $\beta$  and TS1 $\alpha$  were obtained after transfection with human IL-2R $\beta$  and human IL-2R $\alpha$  cDNA, respectively. Both cell lines bind IL-2. mAb H2-8 inhibits the binding of IL-2 to TS1 $\beta$  without significantly 25 affecting IL-2 binding to TS1 $\alpha$  (data not shown).

The binding properties of mAb H2-8 were studied by ELISA. Plates were coated with either IL-2 or peptides 1-22 or 1-30. mAb H2-8 binds to IL-2 and peptide 1-30, but does not recognize peptide 1-22. As control mAb 19B11 (previously characterized) recognized both peptides (Figure 2).

30 Binding inhibition experiments were performed to further characterize the specificity of mAb H2-8. Plates were coated with IL-2 and a concentration of mAb H2-8 giving approximatively 50% of maximum binding was used. H2-8 was preincubated with different peptides including five decapeptides (1-10, 5-15, 10-20, 15-25, 20-30). Only IL-2 and peptide 1-30 were able to inhibit the binding of mAb H2-8 to IL-2. Peptide 35 1-30 was the most efficient inhibitor in these experiments (Figure 2). This result is compatible with the fact that isolated peptide 1-30 folds in an  $\alpha$  helical configuration ( $\alpha$ -

helix content of 50% ± 7%) whereas peptide 1-22 does not (13% ± 5%) as measured by circular dichroism. Therefore peptide 1-30 may adopt some unique structural conformation very close to that of native IL-2. As control the binding of mAb 19B11 is inhibited by IL-2 but also by peptides 1-10, 1-22 and 1-30. This confirms that the epitope of mAb 19B11 is near the NH<sub>2</sub> terminal position of IL-2 as previously suggested (MOREAU et al. (1995b) Mol. Immunol. 32:1047-1056).

5                   Example 2: Peptide synthesis

Peptides were synthesized by the stepwise solid-phase using the Boc/trifluoroacetic acid method (MERRIFIELD (1963) J. Am. Chem. Soc. 85:2149-10 2145), on a *p*-methyl-benzhydrylamine resin (Applied Biosystems) with an Applied Biosystems 430A peptide synthesizer.

10                  Example 3: Involvement of aa at positions 17 and 20 in mAb H2-8 recognition

15                  The reactivity of mAb H2-8 to various IL-2 mutants including one mutant at position 17 (Leu → Asp), four mutants at position 20 (Asp → Asn; Asp → Lys; Asp → Arg and Asp → Leu) and a double mutant 17-20 (Leu17 → Asp and Asp20 → Leu) were tested by Western blot analysis (Figure 3).

20                  mAb H2-8 does not recognize mutations at position 20 or the double mutant (THÈZE J (1994) Eur. Cytokine Netw. 5:353-368; MOREAU et al. (1995a) J. Immunol. 155:3401-3408; CHASTAGNER et al. (1996) Eur. J. Immunol. 26:201-206; MACKAY D (1992) Science 257:410-413). Recognition of the mutation at position 17 is also affected. As positive control the results obtained with mAb 2C4 that recognize an epitope near the NH<sub>2</sub> terminal area of IL-2 are shown. Since this mAb (as 19B11) recognizes peptide 1-10 which bears no mutation, its binding to IL-2 is not affected. Similarly mutations at position 125 and/or 127 do not affect binding to mAbs H2-8 and 2C4, and serve as additional controls. ELISA experiments performed with all the mutants support the data obtained with Western blots (data not shown).

25                  mAb H2-8 as characterized in Example 1 and 19B11 (described in *Molec. Immunol.* (1995) 32:1047-1056) have similar properties: both bind to the NH<sub>2</sub> terminal end of IL-2 and specifically inhibit the binding of IL-2 to IL-2R $\beta$  chain. Since both antibodies recognize sequences located in peptide 1-30 it was of interest to compare the relationship between the corresponding epitopes. Plates coated with mAb H2-8 were used to bind peptide 1-30. The binding of mAb 19B11 to these plates was positive, thus indicating that the epitopes of mAbs H2-8 and 19B11 do not overlap significantly. Various controls performed to verify these results are shown (Figure 3). The binding of 35 19B11 is strictly dependent on the presence of peptide 1-30 and on the coating by mAb

H2-8. Results obtained with mAb 3H9 recognizing the peptide 30-54 further demonstrated the specificity of the data presented in Figure 3.

Example 4: Cell lines, culture media and proliferation assay

TS1 cells express only mouse IL-2R $\gamma$ . TS1 $\beta$  cells were obtained after transfection of TS1 cells with human IL-2R $\beta$  cDNA cloned in the pdKCR expression vector kindly provided by Dr. T. TANIGUCHI (Institute for Molecular and Cellular Biology, Tokyo University, Japan). TS1 $\alpha$  cells were obtained after transfection of TS1 cells with human IL-2R $\alpha$  cDNA cloned in pCMV4 expression vector provided by Drs W.A. KUZIEL and W.C. GREENE (Gladstone Institute Virol./Immunol., San Francisco CA, USA). TS1 $\beta$  and TS1 $\alpha$  were previously characterized (PITTON et al. (1993) Cytokine 5:362-371). CTLL2 and YT were also used for IL-2 binding studies.

All cultures were performed in complete medium composed of RPMI 1640 (BioProducts, Walkerville, MD), 10% heat inactivated FCS (Serovial, Vogelgsun, France), 2 mM glutamine, 100 units/ml penicillin, 100  $\mu$ g/ml streptomycin, 50 mM 2- $\beta$ -mercaptoethanol (2 $\beta$ ME). TS1 $\beta$  and TS1 $\alpha$  cell lines were grown as TS1 cells in complete medium supplemented with supernatant of recombinant baculovirus expressing murine IL-9 proteins (DIB 349) (UYTTENHOVE et al. (1988) Proc. Natl. Acad. Sci. USA 85:6934-6938).

TS1 $\beta$  cells were cultured ( $10^4$  cells/well) in 96 wells in flat-bottomed microtiter plates with a final volume of 0.2 ml. Various concentrations of human rIL-2, IL-2 muteins or mouse rIL-9 were assayed. In order to test the inhibitory effect of mAbs, different concentrations of these reagents were mixed in the culture wells with the respective lymphokines for 30 min at low temperature before adding the cells. The inhibitory effects of mutein 20 Leu (described in *Cytokine* (1997) 9:488-498, which is incorporated herein by reference in its entirety) was studied by preincubating the cells (30 min at 4°C) with the indicated concentration of inhibitor before adding IL-2 or IL-9 to the wells. Cultures were pulsed with 0.5  $\mu$ Ci/well of ( $^3$ H) TdR after 36 h of incubation and harvested 15 h later.

Example 5: Biological properties of mAb H2-8

The biological properties of mAb H2-8 were evaluated on the proliferation of the IL-2- or IL-9 -dependent TS1 $\beta$  cell line (Figure 4). In these experiments the IL-2 mutant IL-2 Pro<sup>125</sup> (Cys → Pro) was used. This mutation slightly reduces the affinity of IL-2 for IL-2R without affecting the maximum proliferation obtained when higher concentrations of mutant are used.

Figure 4 shows that different concentrations of mAb H2-8 reduce the IL-2 proliferation of TS1 $\beta$ . A progressive shift of the IL-2 titration curve is observed with

increasing concentrations of mAb H2-8. Inhibitory effects of mAb H2-8 are comparable to those obtained with mAb 19B11, which was also found to inhibit the proliferation of cells bearing high affinity IL-2R (MOREAU et al. (1995b) Mol. Immunol. 32:1047-1056). Addition of both mAb H2-8 and 19B11 completely abolishes IL-2 proliferation even at a very high dose of IL-2.

As controls Figure 4 shows that mAb 11B11 (specific for mouse IL-4) does not affect the IL-2-dependent proliferation of TS1 $\beta$ . IL-9-induced proliferation of TS1 $\beta$  is also not affected by either H2-8 or 19B11.

Example 6: IL-2 binding assay and inhibition

The IL-2 binding assay was performed as already described (MOREAU et al. (1995b) Mol. Immunol. 32:1047-1056).  $^{125}$ I-labelled IL-2 binding to different cell lines was first studied. Inhibition experiments were performed at concentration of  $^{125}$ I-labelled IL-2 giving between 50 to 70% maximum binding. The effects of the different muteins was analyzed after 1hr preincubation at 4°C followed by incubation with  $^{125}$ I-labelled IL-2 (3hr at 4°C). In each experiments non-specific binding was determined. The data were expressed as % inhibitory capacity of the different mutein versus wild type protein.

Example 7: Physico-chemical properties of peptide IP130 (*Cytokine*, 1997)

The amino terminal peptide of IL-2 including aa 1 to 30 has a molecular weight of 3422.

The circular dichroism studies performed with IP130 indicates that at 20°C, in phosphate buffer (20mM, pH 7.2), 50 % of the residues are in an  $\alpha$  helix configuration.

The quaternary structure of peptide IP130 was also studied by sedimentation - diffusion equilibrium. At concentration above  $5 \times 10^{-6}$  M, most of the molecules are in a tetrameric form (in equilibrium with an octomeric complex).

The aminoacid sequence 1 to 30 shows 7 leucines and 2 isoleucines among the first 20 residues. The periodicity of these aa as well as the above results suggest a structural model for IP130 that would comprise 4 peptides organized in 4  $\alpha$  helices. In this model leucines and isoleucines side chains appear on the same face. This face is hydrophobic and four of these faces would build an hydrophobic core inaccessible to water. At high concentration peptide 1-10 tend to dimerize and this would explain the formation of octameric peptides.

The binding of IP130 to soluble IL-2R $\beta$  chain was studied. Soluble IL-2R $\beta$  has been found to be dimeric in solution. From the results, a structural model has

been proposed : the complex would include four IP130 peptides and two IL-2R $\beta$  chains ((IP130)<sub>4</sub>/(IL-2R $\beta$ )<sub>2</sub>).

Example 8: Biological properties of IP130

Studies were performed either with a murine cell line transfected by  
5 human IL-2R $\beta$  gene (TS1 $\beta$ ) or with an IL-2 dependant human leukemic cell line (Kit 225 from Dr. T. Hori).

IP130 stimulates the proliferation of TS1 $\beta$  in the absence of IL-2. In the presence of IL-2 a strong synergy is observed with the peptide. Both activities are obtained at comparable concentrations (IC-50  $\approx$   $\mu$ M).

10 IP130 acts only on cell lines expressing human IL-2R $\beta$ . This is in agreement with previous studies showing that murine IL-2R $\beta$  does not bind IL-2 (CHASTAGNER et al. 1996, Eur. J. Immunol. 26:201-6). Consequently, classical murine cell lines (C30-1, CTLL, HT-2, ....) usually used to assay IL-2 activity remains insensitive to IP130 effects. Furthermore anti-human IL-2R $\beta$  blocking mAb neutralizes the effects  
15 of IP130.

Alone the peptide induces the phosphorylation of proteins on Kit 225 cell line. On the pattern of phosphorylated proteins, the kinase Shc is easily recognized. After specific immunoprecipitation and blotting with mAb 4G10 (anti-Ptyr), phosphorylated IL-2R $\beta$  is identified on lysates from kit 225 cell line stimulated by IP130.  
20 c-myc induction which depends on IL-2R $\beta$  phosphorylation is also observed after IP130 stimulation. STAT-3 and STAT-5 are not activated after IP130 stimulation since IL-2R $\gamma$  is not involved in IP130 interaction with Kit 225.

Example 9: Immunological properties of IP130 and use as therapeutic agent

IL-2R $\beta$  chain is constitutively expressed by human NK cells from all  
25 donors studied (DAVID et al., Blood 91:165-172, 1998). Monocytes only express IL-2R $\gamma$  chain. Other lymphocytes do not express neither IL-2R $\beta$  nor IL-2R $\alpha$  nor IL-2R $\gamma$  (DAVID et al. 1998).

IP130 induces NK cells to enter into the cell cycle.

IP130 has been also tested on the generation of LAK *in vitro*. IP130  
30 induces LAK activity as tested on K562 targets.

mAb H2-8 was isolated after immunization with IL-2 peptide 1-30. It recognizes both IL-2 and peptide 1-30, but not the shorter peptides covering the same region (Figure 2). This result suggests that mAb H2-8 recognizes a conformational epitope on the N-terminal region of IL-2, and that this epitope is mimicked by the 1-30 peptide. Indeed, circular dichroism measurements reveal a significant fraction of  $\alpha$ -helical structure for the 1-30 peptide. Furthermore, H2-8 can bind to peptide 1-30 even in the  
35

presence of mAb 19B11 (which recognizes a linear epitope within the non-helical part of the peptide 1-30), but does not recognize IL-2 mutants at position 20 (in the center of  $\alpha$ -helix A) as determined by Western blot analysis or ELISA (Figure 3 and other data not shown). The antibody also inhibits the bioactivity of IL-2 on TS1 $\beta$  cells (Figure 4), whose proliferation is strictly dependent on the expression of the human IL-2R $\beta$  chain. Taken together, these results demonstrate that mAb H2-8 recognizes an epitope around Asp20 of IL-2, a region that directly influences the interaction of the cytokine with IL-2R $\beta$ .

Having now fully described the invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

CLAIMS

1. An antibody which binds to a peptide consisting of SEQ ID NO.: 2 or SEQ ID NO.: 4.
2. The antibody of Claim 1, wherein said antibody is a monoclonal antibody.
3. The antibody of Claim 1, wherein said antibody is a polyclonal antibody.
4. The antibody of Claim 1, wherein said antibody is produced by hybridoma H2-8.
5. A DNA sequence encoding a peptide consisting of SEQ ID NO.: 2 or SEQ ID NO.: 4.
6. A method of detecting *in vitro* the presence or activity of IL-2R, wherein said IL-2R is measured by:
  - a) contacting (1) a biological sample from a mammal in which the presence or activity of said IL-2R is suspected with (2) a peptide which binds to the antibody of Claim 1 under the conditions that allow binding of said IL-2R to said peptide to occur; and
  - b) detecting whether binding has occurred between said IL-2R from said sample and the peptide which binds to the antibody of Claim 1.
7. A method for inhibiting the activity of an IL-2R comprising contacting said IL-2R with an amount of the peptide which binds to antibody of Claim 1 sufficient to inhibit binding of IL-2 to said IL-2R under conditions that allow binding of said peptide to said IL-2R to occur.
8. A method of inhibiting the activity of an IL-2R comprising contacting said IL-2R with an amount of the antibody of Claim 1 sufficient to inhibit bind of IL-2 to said IL-2R under conditions that allow binding of said peptide to said IL-2R to occur.
9. Use of a peptide comprising of SEQ ID NO.: 2 or SEQ ID NO.: 4 for the preparation of a medicament useful to induce in a patient selected useful activities of IL-2.
10. A vector containing the DNA sequence of Claim 5.
11. Use of the vector of Claim 10 for the preparation of a medicament useful to treat a patient deficient in IL-2 activity.
12. The use of Claim 9 wherein said peptide comprising of SEQ ID NO.: 2 or SEQ ID NO.: 4 is included in an admixture comprising a cytokine.

13. The use of Claim 9 wherein said peptide comprising of SEQ ID NO.: 2 or SEQ ID NO.: 4 is in an amount able to induce said useful activities.

14. The use of Claim 12 wherein the cytokine is IL-2, IL-4, IL-9, IL-7 or IL-15.

5 15. The use of Claim 14 wherein the amount of IL-2, administered per injection, is  $1 \times 10^6$  international units.

10 16. A peptide, which is IP130, having SEQ ID NO.: 2 or SEQ ID NO.: 4 or a homologous sequence thereof which differs from SEQ ID NO.: 2 or SEQ ID NO.: 4 by one or more conservative changes, wherein said homologous sequence exhibits substantially the same activity or binding characteristics or both as SEQ ID NO.: 2 or SEQ ID NO.: 4.

17. The peptide of Claim 16, which is IP 130, having SEQ ID NO.: 2 or SEQ ID NO.: 4.

15 18. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4 having a conservative change of non-polar R-groups by other non-polar R groups in amino acids thereof.

19. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, having a conservative change of uncharged polar R groups by other uncharged polar R groups in amino acids thereof.

20 20. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, having a conservative change of charged polar R groups by other charged polar R groups in amino acids thereof.

25 21. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2, or SEQ ID NO.: 4 wherein Lys is substituted for Arg, or vice versa so that a positive charge is maintained.

22. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, wherein Glu is substituted for Asp, or vice versa so that a negative charge is maintained.

30 23. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, wherein Ser is substituted for Thr, such that a free-OH group is maintained.

24. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, wherein Gln is substituted for Asn such that a free-NH<sub>2</sub> group is maintained.

25. The peptide of Claim 16, which is a homologous sequence of SEQ ID NO.: 2 or SEQ ID NO.: 4, wherein said activity comprises induction of SHC phosphorylation; or induction of the SHC/MAPK pathway.

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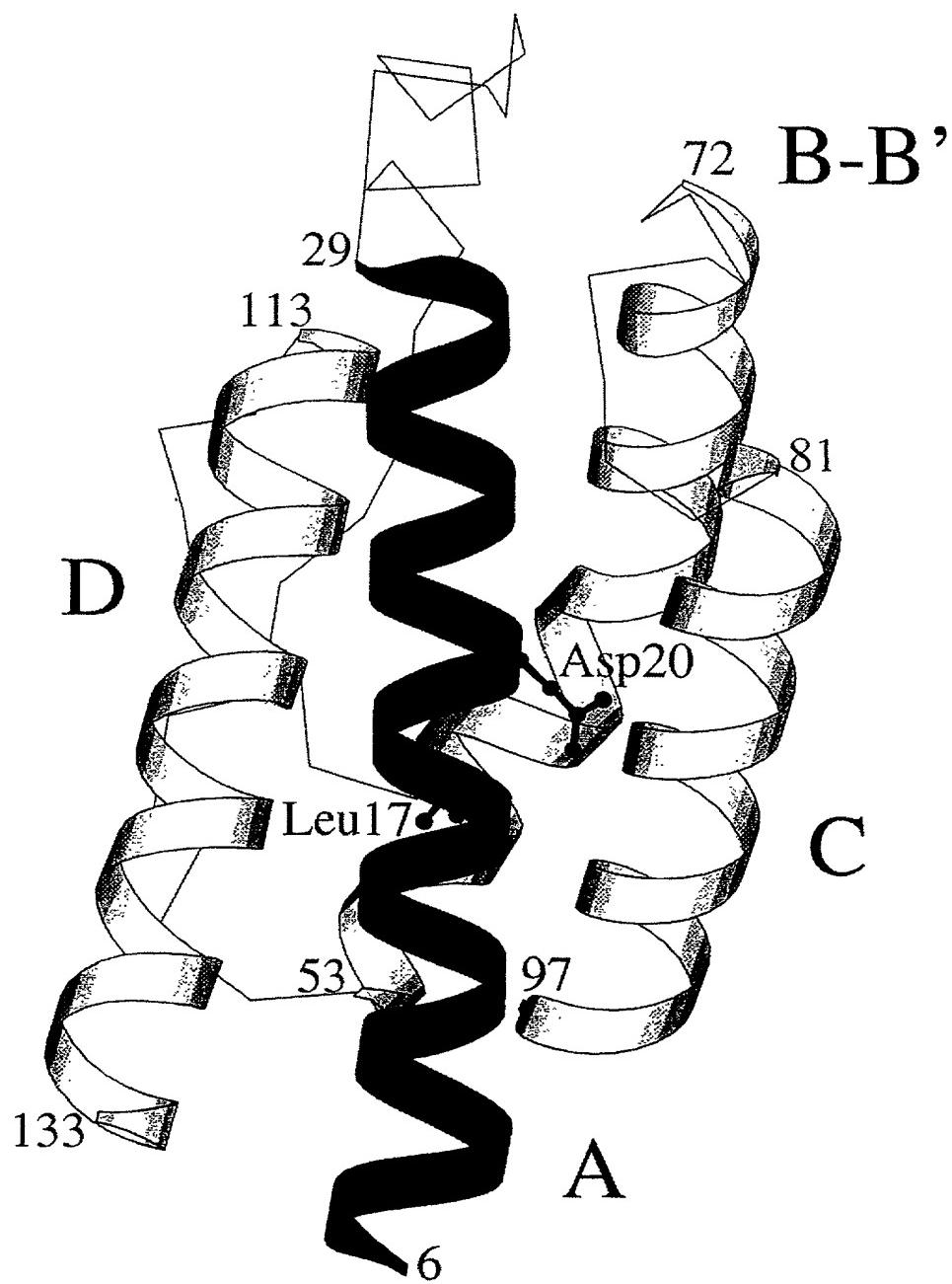


FIGURE 1

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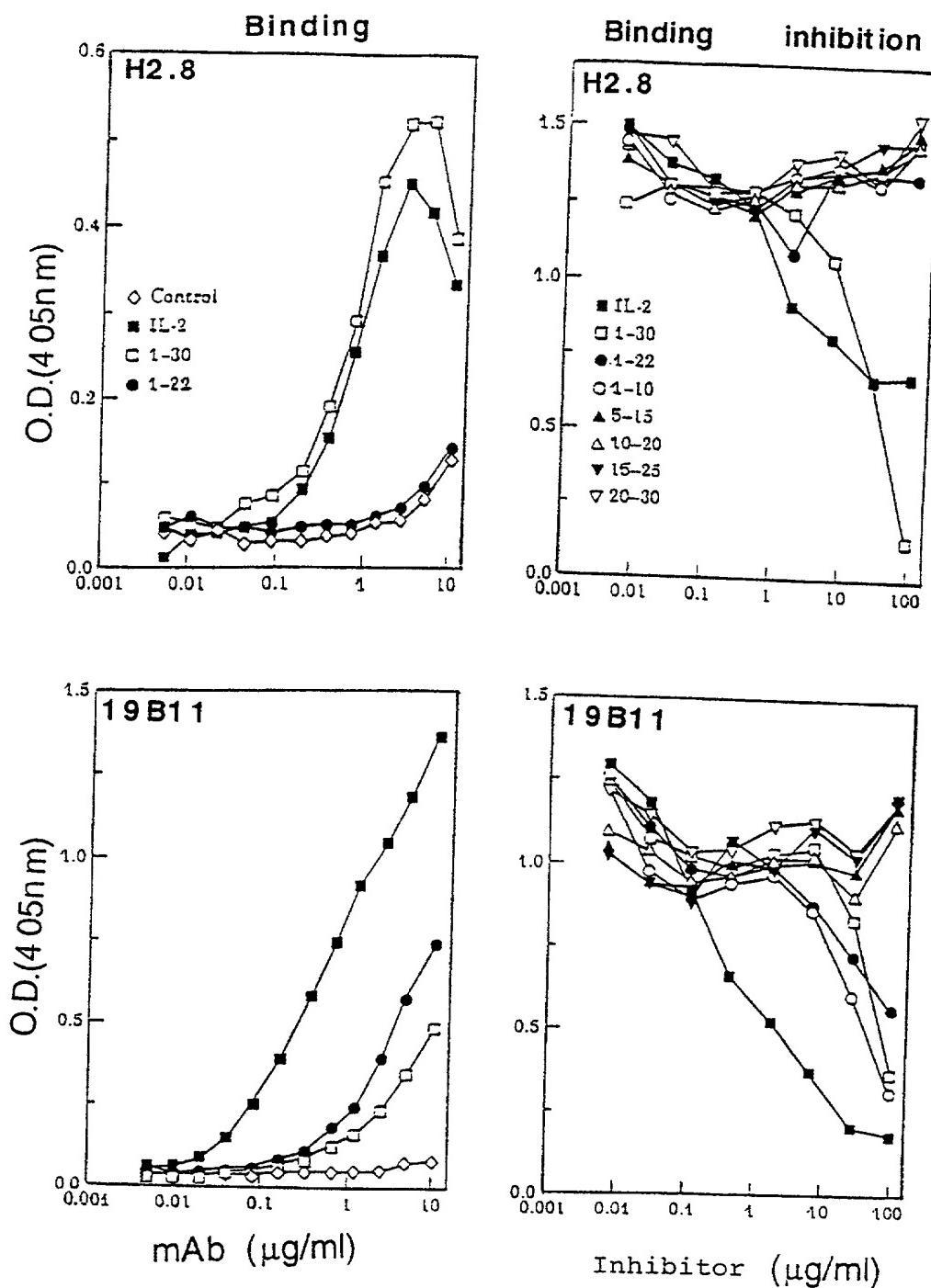


FIGURE 2

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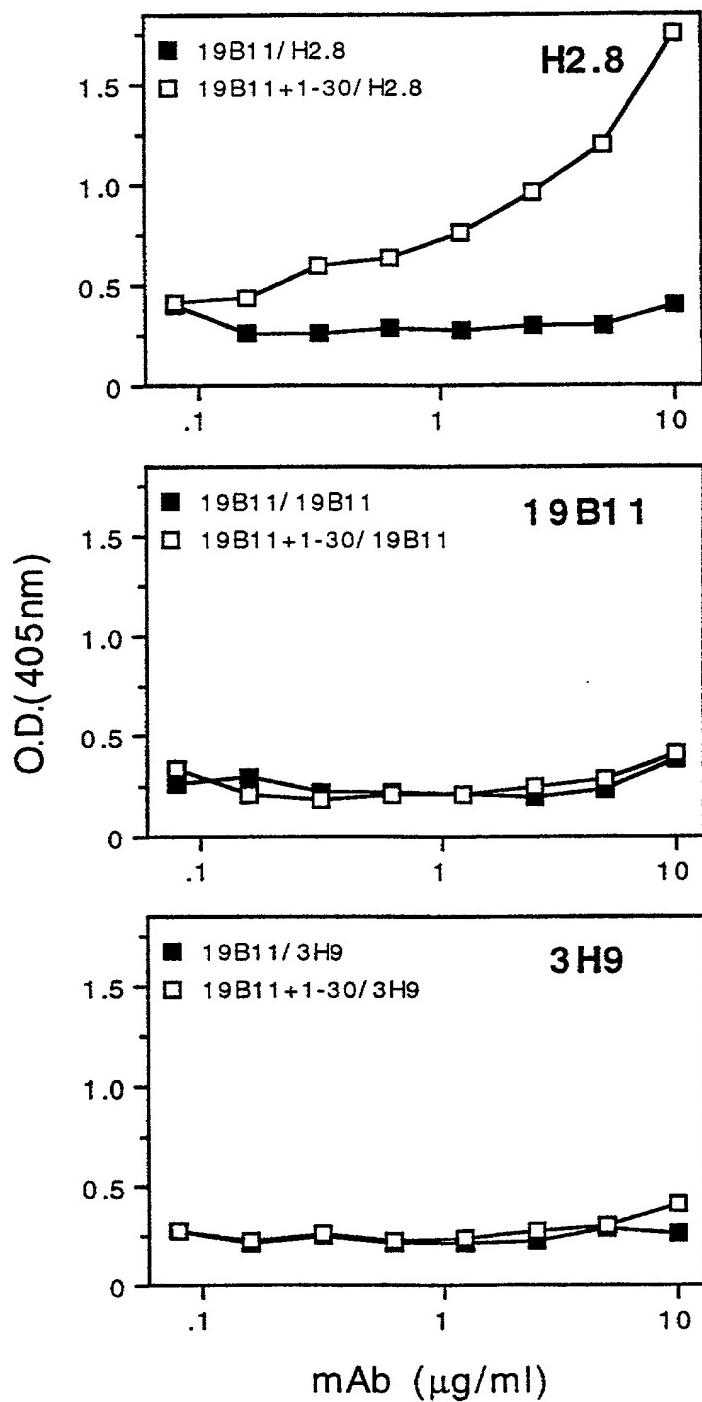


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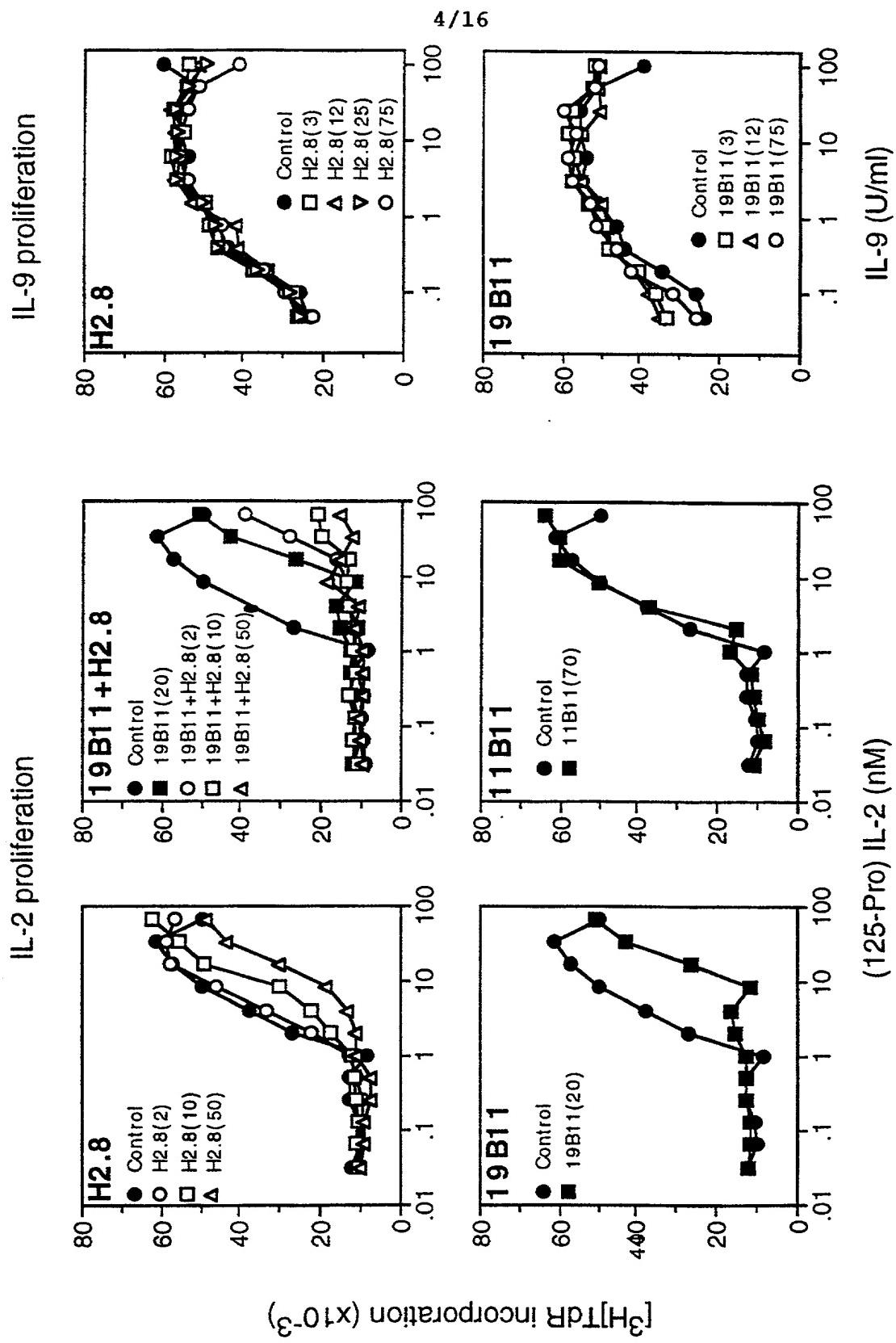


FIGURE 4

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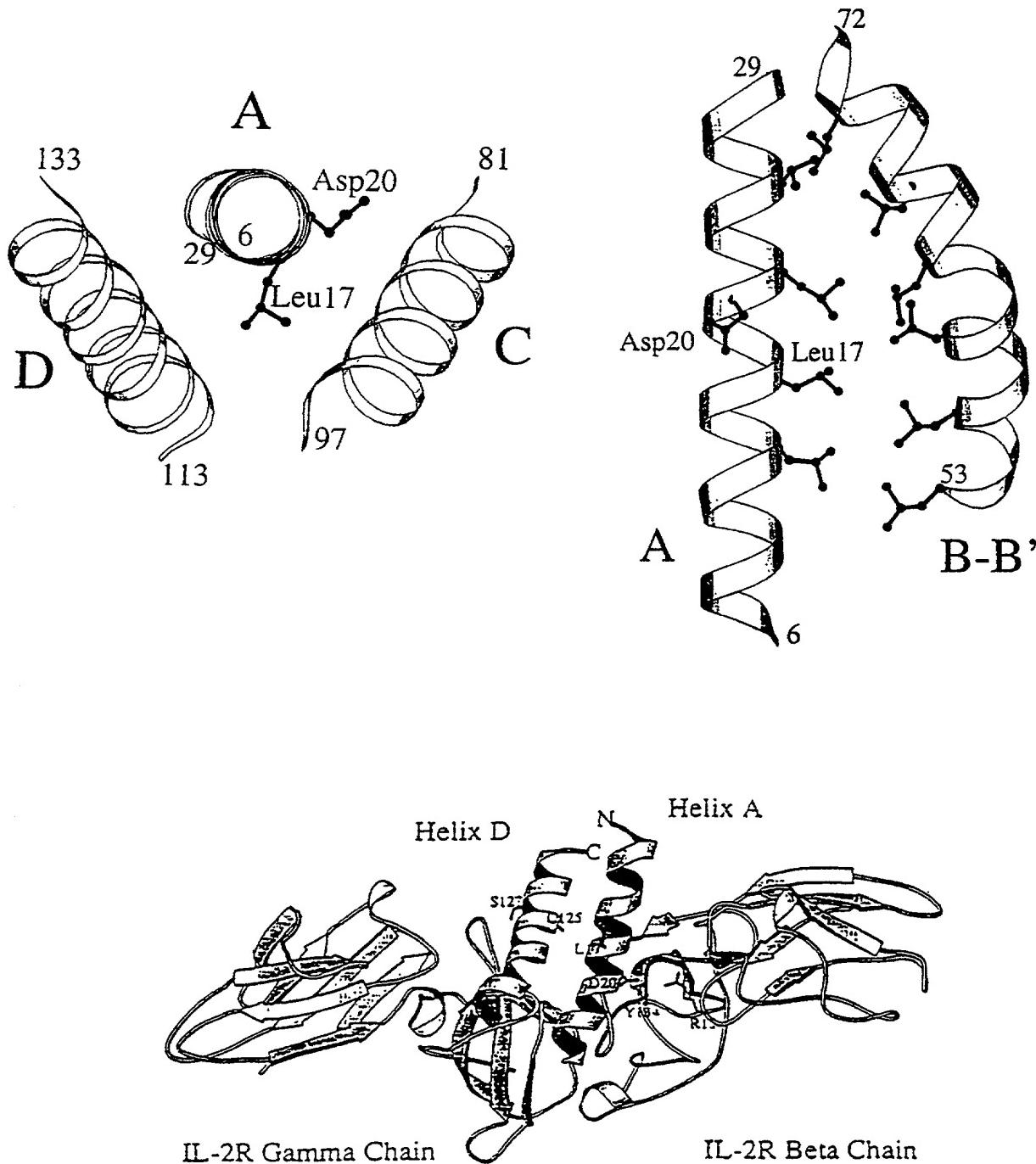


FIGURE 5

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## Interleukine-2 receptor

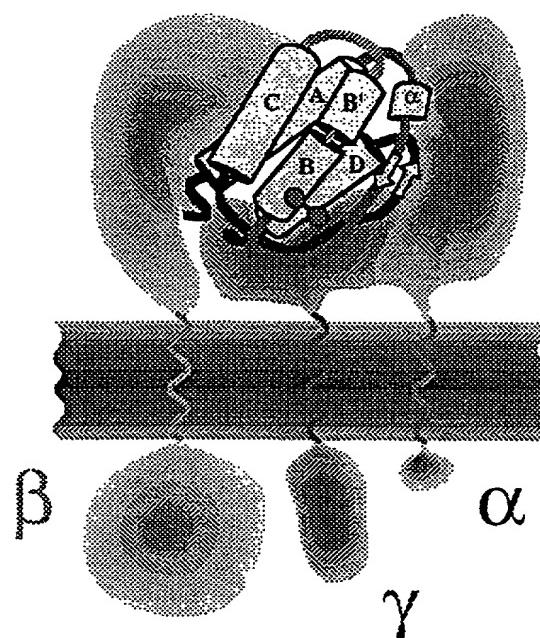
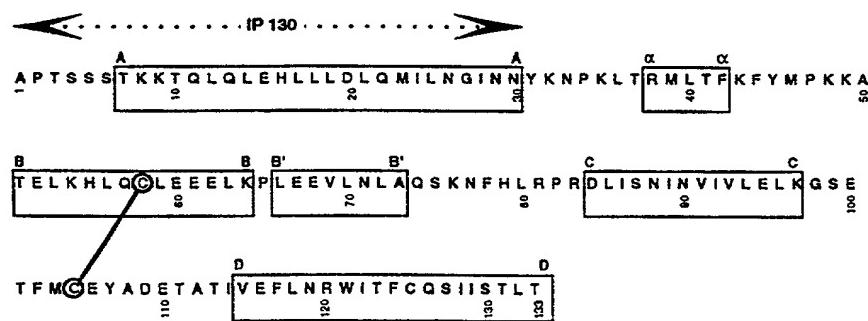
IL-2 AND IP130 SEQUENCE ( $\alpha$ -HELICES ARE BOXED)

FIGURE 6

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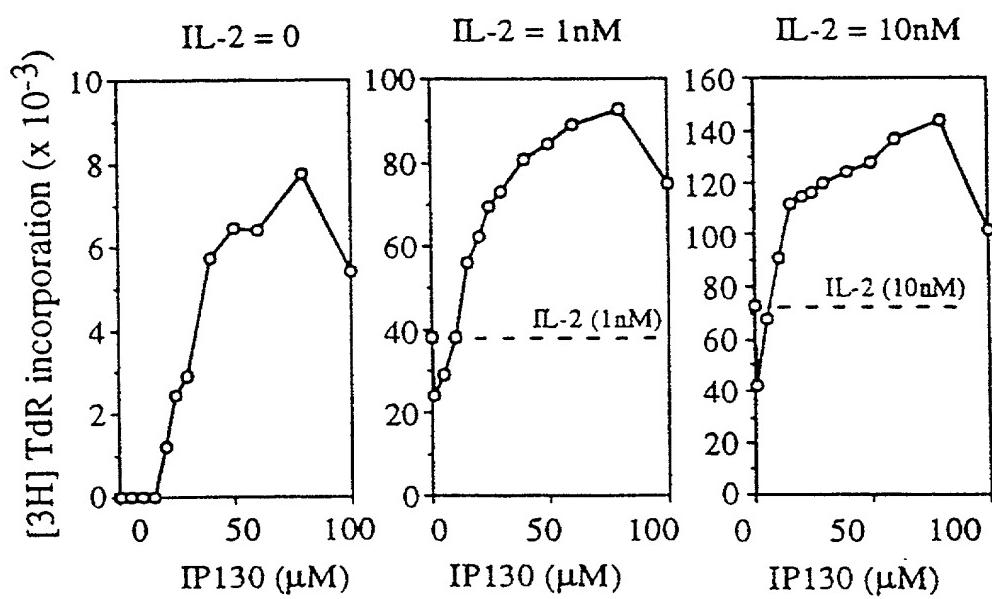


FIGURE 7

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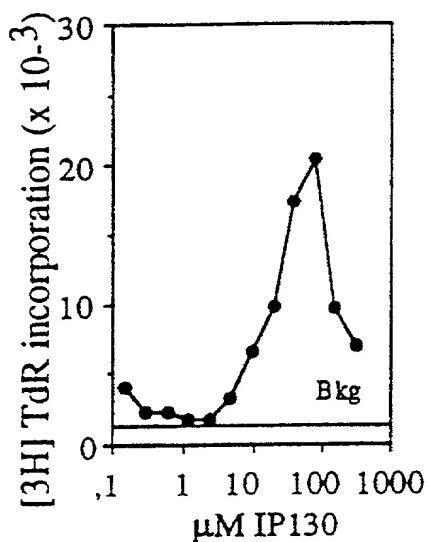


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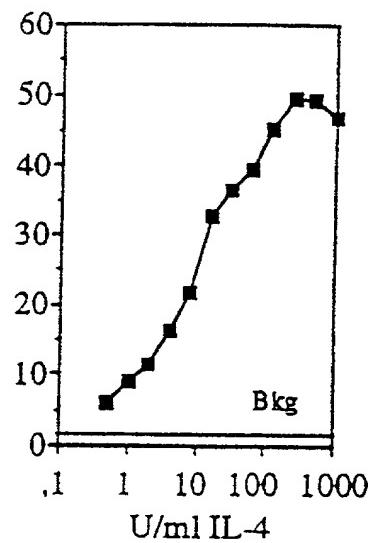


FIGURE 8B

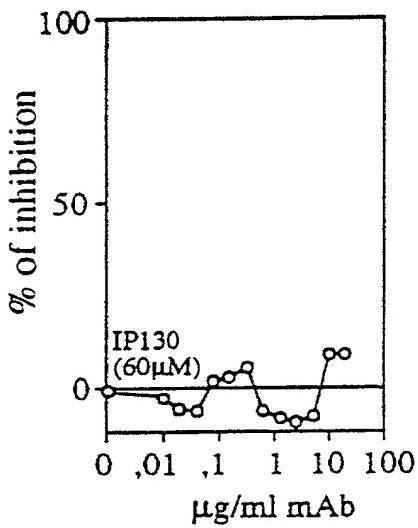


FIGURE 8C

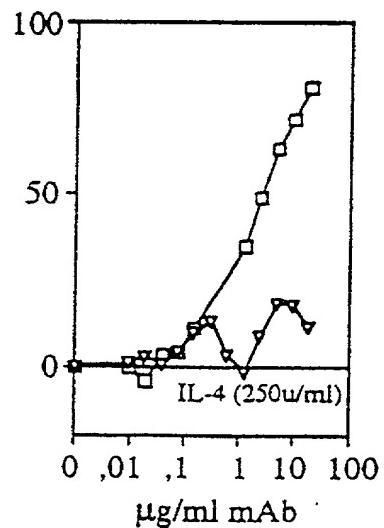
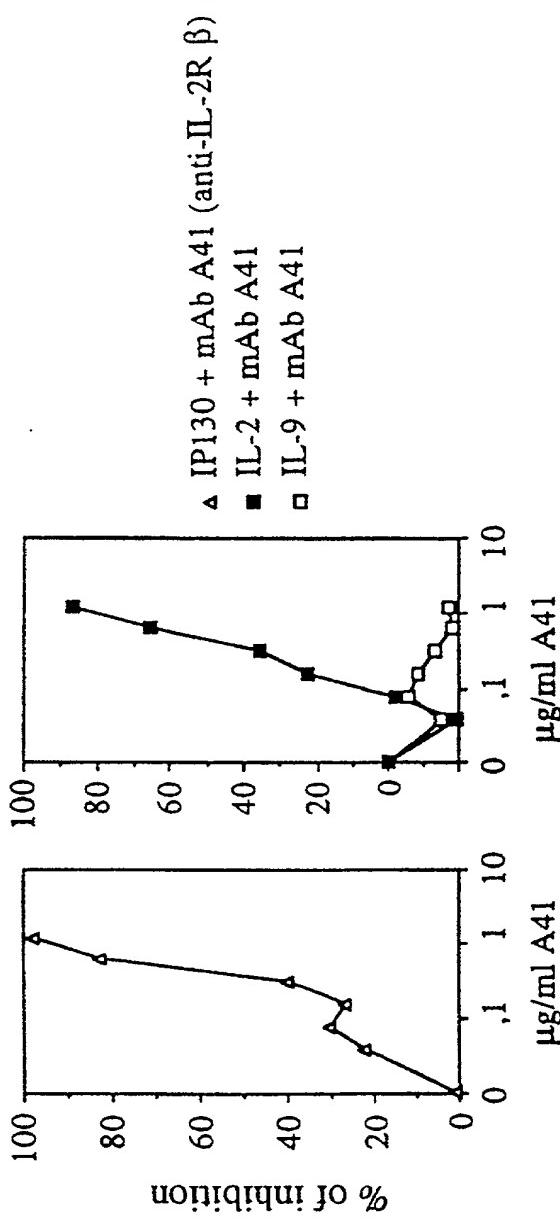
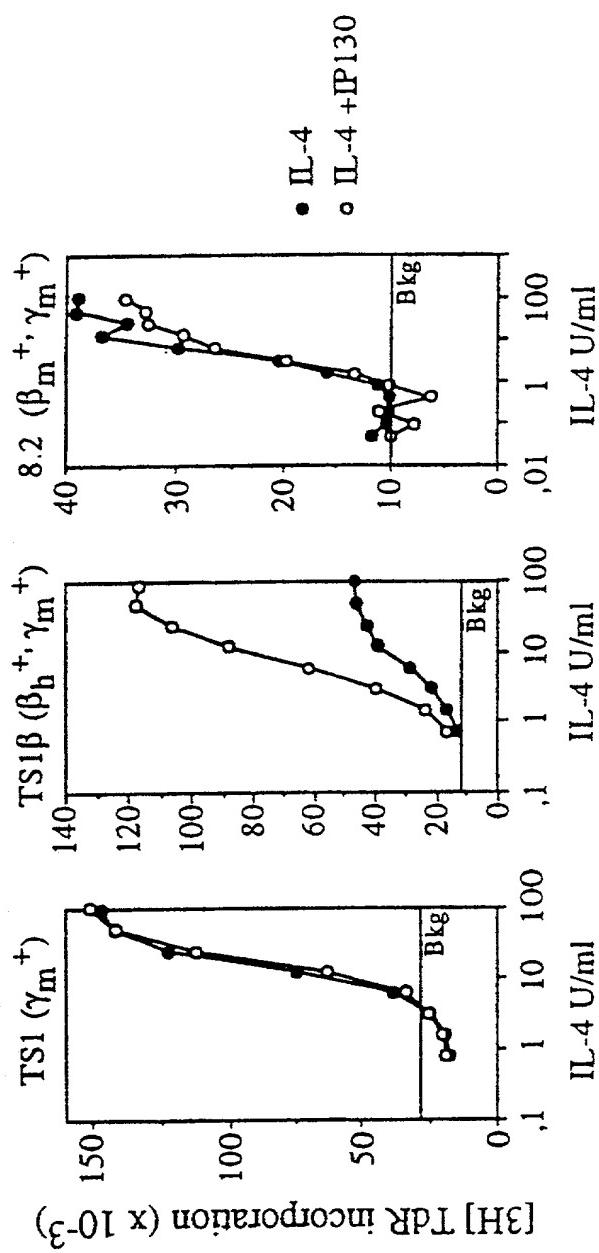


FIGURE 8D

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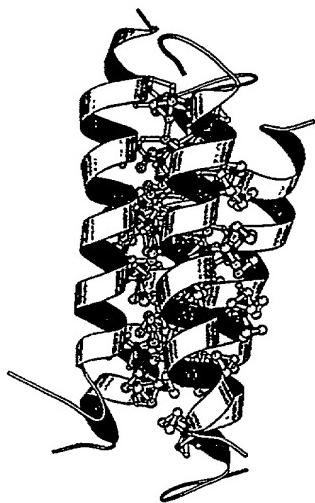


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	% helix	Main molecular species	Activity
	(Circular dichroism)		
1	30 35% (4μM)	Tetramer (4M-8M, Kd=30-100μM) Octamer	+ + +
10	30	22% (150 Å 30μM)	+ + +
1	22	<2%	
1	10	0%	
5	15	0%	
10	20	Dimer (1M-2M, Kd=0.2μM) Tetramer (2M-4M, Kd=100μM)	
20	30	Dimer (1M-2m, Kd=113μM)	
		Monomer	ND
		Monomer	+

FIGURE 10

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IP130

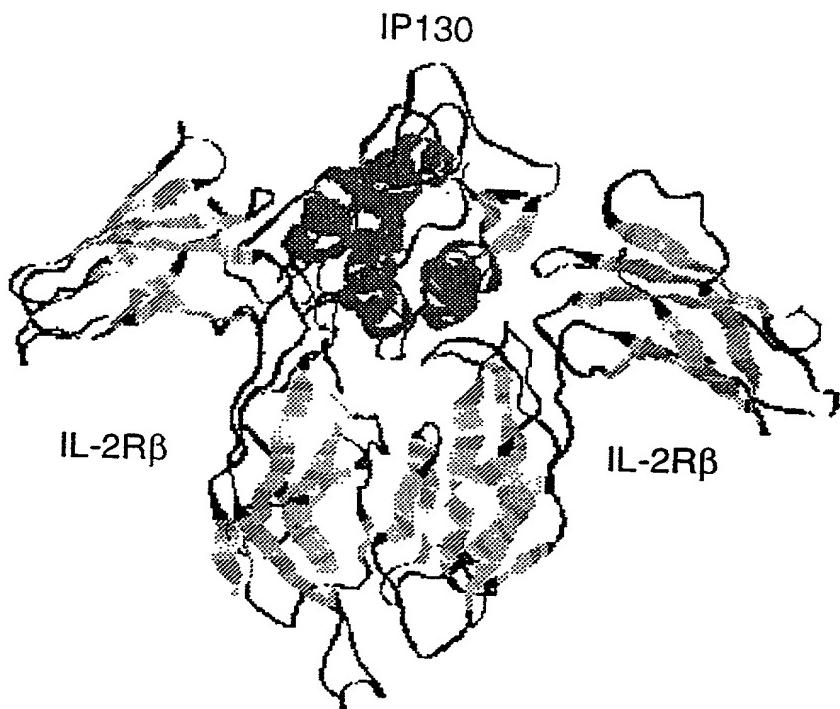


FIGURE 11

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BLOT 4G10 (ANTI  
PHOSPHOTYROSINE)

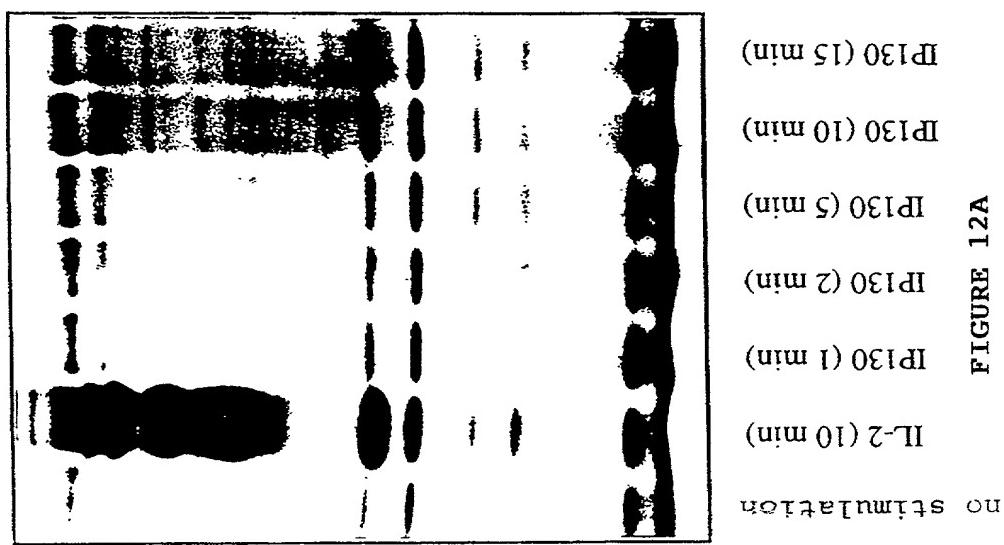


FIGURE 12A

BLOT ANTI SHC

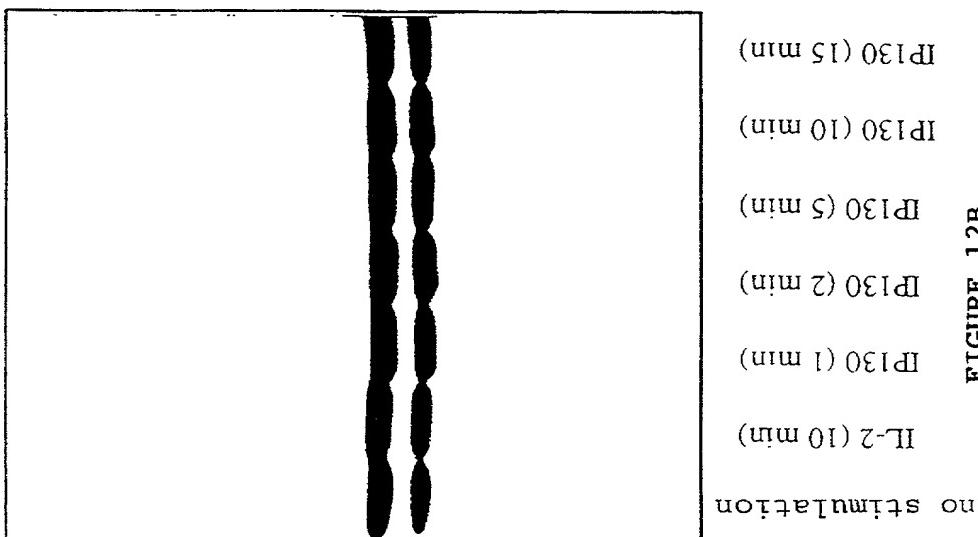


FIGURE 12B

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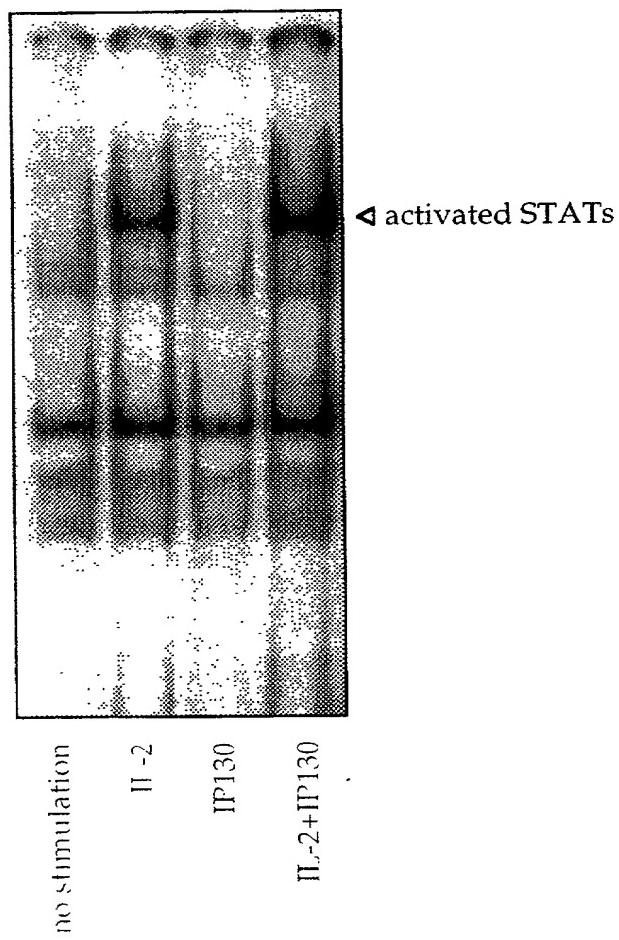
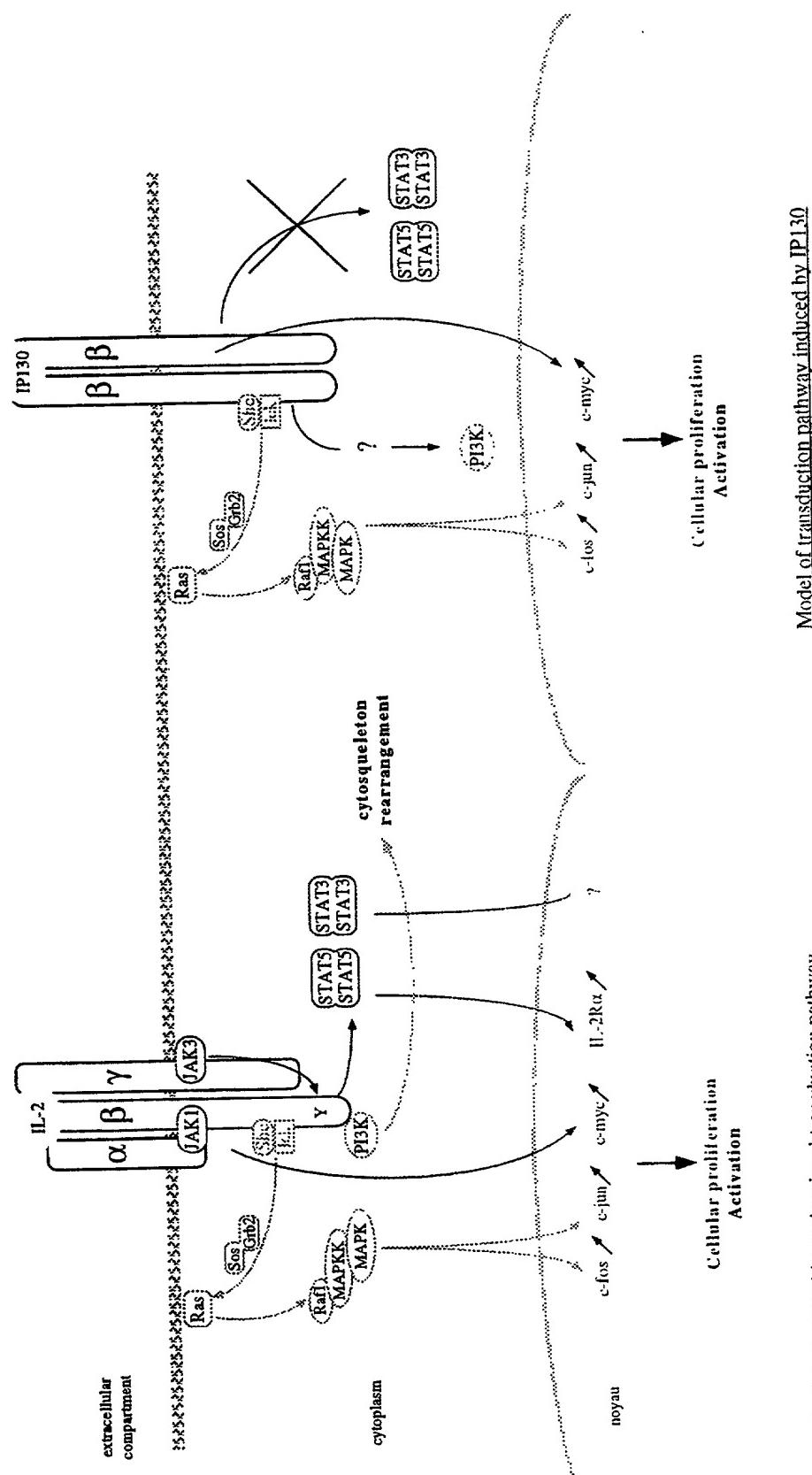


FIGURE 13

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Model of transduction pathway induced by IP130

IL-2 receptor and its major signal transduction pathway

FIGURE 14

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NK cells (CD56<sup>+</sup>) entering in S+G2/M phases after IP130 stimulation  
(synergy with IL-2)

Treatment	J31	J32	J33
IL-2 50 nM	14	12	14
IP130 60μM	0	17	≤5
IP130 120μM	0	14	<5
IL-2 50 nM + IP130 60μM	26	21	7
IL-2 50 nM + IP130 120μM	28	28	28

FIGURE 15

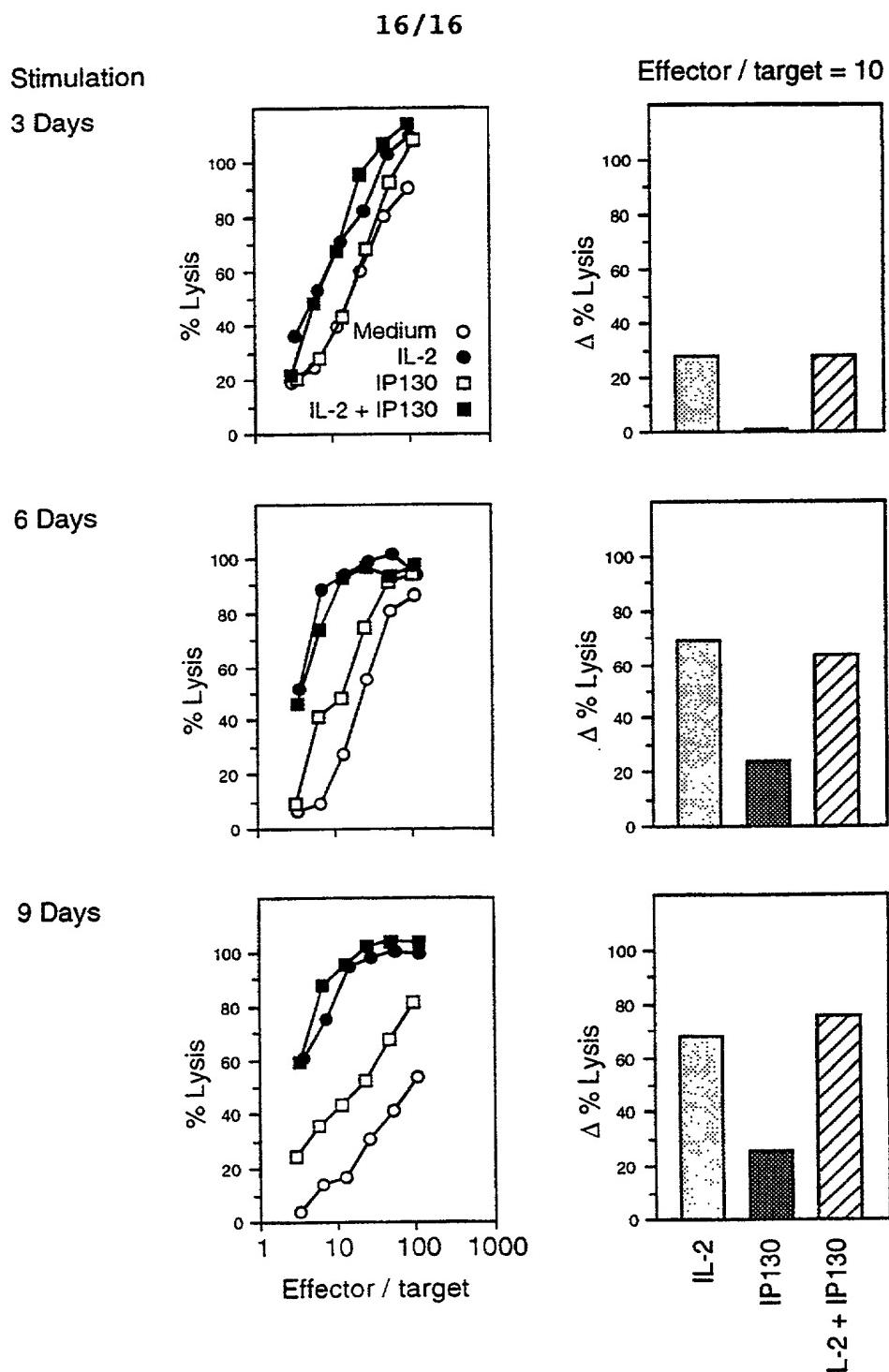


FIGURE 16

**Declaration and Power of Attorney for Patent Application**  
**Déclaration et Pouvoirs pour Demande de Brevet**  
**French Language Declaration**

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

- ci-joint
- a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnaiss devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

**Peptides of IL-2 and derivatives thereof and their use as therapeutic agents**

the specification of which :

- is attached hereto.
- was filed on

as United States Application Number or PCT International Application Number.  
**PCT/IB99/01424 filed on July 16, 1999**

and was amended on  
(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations,§ 1.56.

## French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

Prior Foreign application(s)  
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Numéro)	(Country) (Pays)	USA
<b>09/116,594</b>		
(Number) (Numéro)	(Country) (Pays)	

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
--------------------------------------	----------------------------------

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365C du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
--------------------------------------	----------------------------------

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
--------------------------------------	----------------------------------

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, vérifique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour vérifiable ; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority claimed  
Droit de priorité  
revendiqué

(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	<input checked="" type="checkbox"/> Yes Oui	<input type="checkbox"/> No Non
<b>16.07.1998</b>		
(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	<input type="checkbox"/> Yes Oui	<input type="checkbox"/> No Non

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
--------------------------------------	----------------------------------

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Status) (patented, pending, abandoned)  
(Statut) (breveté, en cours d'examen, abandonné)

(Status) (patented, pending, abandoned)  
(Statut) (breveté, en cours d'examen, abandonné)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## French Language Declaration

**POUVOIRS :** En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques : (mentionner le nom et le numéro d'enregistrement).

**POWER OF ATTORNEY :** As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith : (list name and number)

Norman F.Oblon, Reg. No. 24,618 ; Marvin J. Spivak, Reg. No. 24,913 ; C. Irvin McClelland, Reg. No. 21,124 ; Gregory J. Maier, Reg. No. 25,599 ; Arthur I. Neustadt, Reg. No. 24,854 ; Richard D. Kelly, Reg. No. 27,757 ; James D. Hamilton, Reg. No. 28,421 ; Eckhard H. Kuesters, Reg. No. 28,870 ; Robert T. Pous, Reg. No. 29,099 ; Charles L. Gholz, Reg. No. 26,395 ; William E. Beaumont, Reg. No. 30,996 ; Jean-Paul Lavallee, Reg. No. 31,451 ; Stephen G. Baxter, Reg. No. 34,884 ; Richard L. Treanor, Reg. No. 36,379 ; Stephen P. Wehrhouch, Reg. No. 32,829 ; John T. Goolkasian, Reg. No. 26,142 ; Richard L. Cinn, Reg. No. 34,305 ; Stephen E. Lipman, Reg. No. 30,011 ; Carl E. Shlier, Reg. No. 34,426 ; James J. Kubaski, Reg. No. 34,648 ; Richard A. Neifeld, Reg. No. 35,299 ; J. Derek Mason, Reg. No. 35,270 ; Surinder Sachar, Reg. No. 34,423 ; Christina M. Gadiano, Reg. No. 37,628 ; Jeffrey B. McIntyre, Reg. No. 36,867 ; William T. Enos, Reg. No. 33,128 ; Michael E. McCabe, Jr., Reg. No. 37,182 ; Bradley D. Lytle, Reg. No. 40,073 ; and Michael R. Asey, Reg. No. 40,294, with full powers of substitution and revocation.

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Direct Telephone calls to : (name and telephone number)

(703) 413-3000

Nom complete de l'unique ou premier inventeur  
**Jacques THEZE**

Full name of sole or first inventor

Signature de l'inventeur

Date

Date

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Residence

Nationalité  
Française

Citizenship

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Post Office Address

Nom complete du second co-inventeur, le cas échéant  
**Ralph ECKENBERG**

Full name of second joint inventor, if any

Signature de l'inventeur

Date

Date

Domicile  
F-78100 Saint Germain en Laye (FRANCE)

Residence

Nationalité  
Française

Citizenship

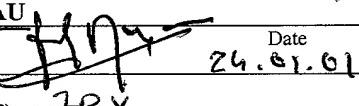
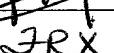
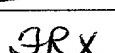
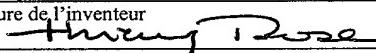
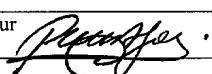
Adresse Postale  
27, rue A. Dumas F-78100 Saint Germain en Laye (FRANCE)

Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

## French Language Declaration

3-00		Nom complete du troisième co-inventeur, le cas échéant <u>Jean-Louis MOREAU</u>		Full name of third joint inventor, if any
		Signature de l'inventeur 	Date 24.61.01	Third inventor's signature Date
		Domicile F-75015 Paris (FRANCE)		Residence
		Nationalité Française		Citizenship
		Adresse Postale 7, rue Falguière, F-75015 Paris (FRANCE)		Post Office Address
4-00		Nom complete du quatrième co-inventeur, le cas echeant <u>Michel GOLBERG</u>		Full name of fourth joint inventor, if any
		Signature de l'inventeur 	Date 21.01.01	Fourth inventor's signature Date
		Domicile F-75724 Paris Cedex 15 (FRANCE)		Residence
		Nationalité Française		Citizenship
		Adresse Postale 25-28, rue du Docteur Roux F-75724 Paris Cedex 15 (FRANCE)		Post Office Address
5-00		Nom complete du cinquième co-inventeur, le cas echeant <u>Thierry ROSE</u>		Full name of fifth joint inventor, if any
		Signature de l'inventeur 	Date 01/19/01	Fifth inventor's signature Date
		Domicile F-75724 Paris Cedex 15		Residence
		Nationalité Française		Citizenship
		Adresse Postale 25-28, rue du Docteur Roux F-75724 Paris Cedex 15 (FRANCE)		Post Office Address
6-00		Nom complete du sixième co-inventeur, le cas echeant <u>Pedro ALZARI</u>		Full name of sixth joint inventor, if any
		Signature de l'inventeur 	Date 24/1/01	Sixth inventor's signature Date
		Domicile F-75015 Paris (FRANCE)		Residence
		Nationalité Italienne		Citizenship
		Adresse Postale 141, rue de Vaugirard F-75015 Paris (FRANCE)		Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

## French Language Declaration

Nom complete du septième co-inventeur, le cas échéant <b>Jean-Claude MAZIE</b>	Full name of seventh joint inventor, if any	
Signature de l'inventeur <i>Jean-Claude MAZIE</i>	Date <i>16/01/01</i>	Third inventor's signature
Domicile F-92600 Asnières (FRANCE) <i>JRX</i>	Residence	
Nationalité Française	Citizenship	
Adresse Postale 24, rue des Jardins, F-92600 Asnières (FRANCE)	Post Office Address	
Nom complete du huitième co-inventeur, le cas écheant	Full name of eighth joint inventor, if any	
Signature de l'inventeur	Date	Fourth inventor's signature
Domicile	Residence	
Nationalité	Citizenship	
Adresse Postale	Post Office Address	
Nom complete du neuvième co-inventeur, le cas écheant	Full name of ninth joint inventor, if any	
Signature de l'inventeur	Date	Fifth inventor's signature
Domicile	Residence	
Nationalité	Citizenship	
Adresse Postale	Post Office Address	
Nom complete du dixième co-inventeur, le cas écheant	Full name of tenth joint inventor, if any	
Signature de l'inventeur	Date	Sixth inventor's signature
Domicile	Residence	
Nationalité	Citizenship	
Adresse Postale	Post Office Address	

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

## SEQUENCE LISTING

<110> INSTITUT PASTEUR  
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ECKENBERG, Ralph  
MOREAU, Jean-Louis  
GOLDBERG, Michel  
ROSE, Thierry  
ALZARI, Pedro  
MAZIE, Jean-Claude

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